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(54) Title: NOVEL MONOCOTYLEDONOUS PLANT GENES AND USES THEREOF

(57) Abstract: Homologues of the *Arabidopsis NIM1* gene, which is involved in the signal transduction cascade leading to systemic acquired resistance (SAR), are isolated from monocotyledonous crops such as *Triticum aestivum* (wheat) and *Oryza sativa* (rice). The invention further concerns transformation vectors and processes for expressing the monocotyledonous *NIM1* homologues in transgenic plants to increase SAR gene expression and enhance broad spectrum disease resistance.

NOVEL MONOCOTYLEDONOUS PLANT GENES AND USES THEREOF

The present invention relates to broad-spectrum disease resistance in plants, including the phenomenon of systemic acquired resistance (SAR). More particularly, the present invention relates to the identification, isolation and characterization of monocotyledonous homologues of the *NIM1* gene involved in the signal transduction cascade leading to systemic acquired resistance in plants.

Plants are constantly challenged by a wide variety of pathogenic organisms including viruses, bacteria, fungi, and nematodes. Crop plants are particularly vulnerable because they are usually grown as genetically-uniform monocultures; when disease strikes, losses can be severe. However, most plants have their own innate mechanisms of defense against pathogenic organisms. Natural variation for resistance to plant pathogens has been identified by plant breeders and pathologists and bred into many crop plants. These natural disease resistance genes often provide high levels of resistance to or immunity against pathogens.

Systemic acquired resistance (SAR) is one component of the complex system plants use to defend themselves from pathogens (Hunt and Ryals, 1996; Ryals *et al.*, 1996). *See also,* U.S. Patent No. 5,614,395. SAR is a particularly important aspect of plant-pathogen responses because it is a pathogen-inducible, systemic resistance against a broad spectrum of infectious agents, including viruses, bacteria, and fungi. When the SAR signal transduction pathway is blocked, plants become more susceptible to pathogens that normally cause disease, and they also become susceptible to some infectious agents that would not normally cause disease (Gaffney *et al.*, 1993; Delaney *et al.*, 1994; Delaney *et al.*, 1995; Delaney, 1997; Bi *et al.*, 1995; Mauch-Mani and Slusarenko, 1996). These observations indicate that the SAR signal transduction pathway is critical for maintaining plant health.

Conceptually, the SAR response can be divided into two phases. In the initiation phase, a pathogen infection is recognized, and a signal is released that travels through the phloem to distant tissues. This systemic signal is perceived by target cells, which react by expression of both SAR genes and disease resistance. The maintenance phase of SAR

refers to the period of time, from weeks up to the entire life of the plant, during which the plant is in a quasi steady state, and disease resistance is maintained (Ryals *et al.*, 1996).

Salicylic acid (SA) accumulation appears to be required for SAR signal transduction. Plants that cannot accumulate SA due to treatment with specific inhibitors, epigenetic repression of phenylalanine ammonia-lyase, or transgenic expression of salicylate hydroxylase, which specifically degrades SA, also cannot induce either SAR gene expression or disease resistance (Gaffney *et al.*, 1993; Delaney *et al.*, 1994; Mauch-Mani and Slusarenko, 1996; Maher *et al.*, 1994; Pallas *et al.*, 1996). Although it has been suggested that SA might serve as the systemic signal, this is currently controversial and, to date, all that is known for certain is that if SA cannot accumulate, then SAR signal transduction is blocked (Pallas *et al.*, 1996; Shulaev *et al.*, 1995; Vernooij *et al.*, 1994).

Recently, *Arabidopsis* has emerged as a model system to study SAR (Uknes *et al.*, 1992; Uknes *et al.*, 1993; Cameron *et al.*, 1994; Mauch-Mani and Slusarenko, 1994; Dempsey and Klessig, 1995). It has been demonstrated that SAR can be activated in *Arabidopsis* by both pathogens and chemicals, such as SA, 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) (Uknes *et al.*, 1992; Vernooij *et al.*, 1995; Lawton *et al.*, 1996). Following treatment with either INA or BTH or pathogen infection, at least three pathogenesis-related (PR) protein genes, namely, PR-1, PR-2, and PR-5 are coordinately induced concomitant with the onset of resistance (Uknes *et al.*, 1992, 1993). In tobacco, the best characterized species, treatment with a pathogen or an immunization compound induces the expression of at least nine sets of genes (Ward *et al.*, 1991). Transgenic disease-resistant plants have been created by transforming plants with various SAR genes (U.S. Patent No. 5,614,395).

Although most of the studies on SAR have been conducted in dicotyledonous plants, SAR has been demonstrated in monocotyledonous plants as well. For example, SAR has been demonstrated in rice, where an inducing infection by *P.s.* pv *syringae* led to systemic protection against *Pyricularia oryzae* (Smith and Metraux, 1991), the causative agent of leaf blast, and in barley and wheat, where a prior infection by *Erysiphe graminis* led to enhanced protection against *E. graminis*, the causative agent of powdery mildew (Schweizer *et al.*, 1989; Hwang and Heitefuss, 1992). Chemically induced resistance by INA has been described in barley (Kogel *et al.*, 1994; Wasternack *et al.*, 1994). More recently, BTH has been shown to induce acquired resistance in wheat against *E. graminis*, *Puccinia recondita*, and *Septoria* spp., and to induce the accumulation of transcripts from a number of novel

plant genes that are also shown to be induced during pathogen infection (Görlach *et al.*, 1996).

A number of *Arabidopsis* mutants have been isolated that have modified SAR signal transduction (Delaney, 1997) The first of these mutants are the so-called *lsd* (lesions simulating disease) mutants and *acd2* (accelerated cell death) (Dietrich *et al.*, 1994; Greenberg *et al.*, 1994). These mutants all have some degree of spontaneous necrotic lesion formation on their leaves, elevated levels of SA, mRNA accumulation for the SAR genes, and significantly enhanced disease resistance. At least seven different *lsd* mutants have been isolated and characterized (Dietrich *et al.*, 1994; Weymann *et al.*, 1995). Another interesting class of mutants are *cim* (constitutive immunity) mutants (Lawton *et al.*, 1993). *See also,* U.S. Patent No. 5,792,904 and International PCT Application WO 94/16077. Like *lsd* mutants and *acd2*, *cim* mutants have elevated SA and SAR gene expression and resistance, but in contrast to *lsd* or *acd2,* do not display detectable lesions on their leaves. *cpr1* (constitutive expresser of PR genes) may be a type of *cim* mutant; however, because the presence of microscopic lesions on the leaves of *cpr1* has not been ruled out, *cpr1* might be a type of *lsd* mutant (Bowling *et al.*, 1994).

Mutants have also been isolated that are blocked in SAR signaling. *ndr1* (non-race-specific disease resistance) is a mutant that allows growth of both *Pseudomonas syringae* containing various avirulence genes and also normally avirulent isolates of *Peronospora parasitica* (Century *et al.*, 1995). Apparently this mutant is blocked early in SAR signaling. *npr1* (nonexpresser of PR genes) is a mutant that cannot induce expression of the SAR signaling pathway following INA treatment (Cao *et al.*, 1994). *eds* (enhanced disease susceptibility) mutants have been isolated based on their ability to support bacterial infection following inoculation of a low bacterial concentration (Glazebrook *et al.*, 1996; Parker *et al.*, 1996). Certain *eds* mutants are phenotypically very similar to *npr1,* and, recently, *eds5* and *eds53* have been shown to be allelic to *npr1* (Glazebrook *et al.*, 1996). *nim1* (noninducible immunity) is a mutant that supports *P. parasitica* (i.e., causal agent of downy mildew disease) growth following INA treatment (Delaney *et al.*, 1995; U.S. Patent No. 5,792,904). Although *nim1* can accumulate SA following pathogen infection, it cannot induce SAR gene expression or disease resistance, suggesting that the mutation blocks the pathway downstream of SA. *nim1* is also impaired in its ability to respond to INA or BTH, suggesting that the block exists downstream of the action of these chemicals (Delaney *et al.*, 1995; Lawton *et al.*, 1996).

Allelic *Arabidopsis* genes have been isolated and characterized, mutants of which are responsible for the *nim1* and *npr1* phenotypes, respectively (Ryals *et al.*, 1997; Cao *et al.*, 1997). The wild-type *NIM1* gene product is involved in the signal transduction cascade leading to both SAR and gene-for-gene disease resistance in *Arabidopsis* (Ryals *et al.*, 1997). Ryals *et al.*, 1997 also report the isolation of five additional alleles of *nim1* that show a range of phenotypes from weakly impaired in chemically induced PR-1 gene expression and fungal resistance to very strongly blocked. Transformation of the wild-type *NPR1* gene into *npr1* mutants not only complemented the mutations, restoring the responsiveness of SAR induction with respect to PR-gene expression and disease resistance, but also rendered the transgenic plants more resistant to infection by *P. syringae* in the absence of SAR induction (Cao *et al.*, 1997). WO 98/06748 describes the isolation of *NPR1* from *Arabidopsis* and a homologue from *Nicotiana glutinosa*. *See also,* WO 97/49822, WO 98/26082, and WO 98/29537. Furthermore, U.S. Patent Application No. 09/265,149 of Salmeron *et al.* describes the isolation of *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato), *Brassica napus* (oilseed rape), and *Arabidopsis thaliana* homologues of the *NIM1* gene. Therefore, while *NIM1* homologues have been isolated from a number of dicotyledonous plant species, *NIM1* homologues have heretofore not been isolated from any monocotyledonous plant species.

Despite much research and the use of sophisticated and intensive crop protection measures, including genetic transformation of plants, losses due to disease remain in the billions of dollars annually. Therefore, there is a continuing need to develop new crop protection measures based on the ever-increasing understanding of the genetic basis for disease resistance in plants. In particular, there is a need for the identification, isolation, and characterization of *NIM1* homologues from additional species of plants, particularly monocotyledonous plants.

The present invention addresses the aforementioned needs by providing homologues of the *Arabidopsis NIM1* gene from monocotyledonous plant species. In particular, the present invention concerns the isolation of *Triticum aestivum* (wheat) and *Oryza sativa* (rice) homologues of the *NIM1* gene, which encode proteins believed to be involved in the signal transduction cascade responsive to biological and chemical inducers that lead to systemic acquired resistance in plants.

Hence, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence from a monocotyledonous plant that is a homologue of the *NIM1* gene.

In one particular embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes SEQ ID NO:2, 8, 10, 12, 14, 16, 18, or 20.

In another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that comprises an at least 20, 25, 30, 35, 40, 45, or 50 (preferably 20) consecutive base pair portion identical in sequence to an at least 20, 25, 30, 35, 40, 45, or 50 (preferably 20) consecutive base pair portion of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

In yet another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

In still another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Orzya sativa* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

In yet another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Triticum aestivum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with a pair of primers comprising the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence from a monocotyledonous plant that hybridizes to the complement of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19 under stringent hybridization and wash conditions.

The present invention also encompasses a chimeric gene comprising a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, a recombinant vector comprising such a chimeric gene, wherein the vector is capable of being stably transformed into a host, as well as a host stably transformed with such a vector. Preferably, the host is a plant such as one of the following agronomically important crops: rice, wheat, barley, rye, canola, sugarcane, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum, and sugarcane. More preferably, the host is a monocotyledonous plant. The present invention also encompasses seed from a plant of the invention.

Further, the present invention is directed to a method of increasing SAR gene expression in a plant by expressing in the plant a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, wherein the encoded protein is expressed in the transformed plant at higher levels than in a wild type plant. Preferably, the host is a monocotyledonous plant.

In addition, the present invention is directed to a method of enhancing disease resistance in a plant by expressing in the plant a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, wherein the encoded protein is expressed in the transformed plant at higher levels than in a wild type plant. Preferably, the host is a monocotyledonous plant.

Further, the present invention is directed to a PCR primer that is SEQ ID NO:3 or 4.

The present invention also encompasses a method for isolating a *NIM1* homologue involved in the signal transduction cascade leading to systemic acquired resistance in plants comprising amplifying a DNA molecule from a monocotyledonous plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19 or with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6. In a preferred embodiment, the monocotyledonous plant DNA library is a *Oryza sativa* (rice) or *Triticum aestivum* (wheat) DNA library.

SEQ ID NO:1 - Genomic DNA sequence of a *NIM1* homologue (pHW01) from wheat.

SEQ ID NO:2 - Protein sequence of the wheat NIM1 homologue encoded by SEQ ID NO:1.

SEQ ID NO:3 - Oligonucleotide primer KL1.

SEQ ID NO:4 - Oligonucleotide primer KL2.

SEQ ID NO:5 - PCR primer NIM 2B.

SEQ ID NO:6 - PCR primer NIM 2D.

SEQ ID NO:7 - 498 bp *NIM*-like DNA fragment amplified from *Oryza sativa* (Rice A), which is a consensus of 13 sequences and has 59% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:8 - Protein sequence encoded by SEQ ID NO:7.

SEQ ID NO:9 - 498 bp *NIM*-like DNA fragment amplified from *Oryza sativa* (Rice B), which has 62% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:10 - Protein sequence encoded by SEQ ID NO:9.

SEQ ID NO:11 - 498 bp *NIM*-like DNA fragment amplified from *Triticum aestivum* (Wheat), which is a consensus of 3 sequences and has 55% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:12 - Protein sequence encoded by SEQ ID NO:11.

SEQ ID NO:13 - Full length cDNA sequence of a *NIM1* homologue from *Oryza sativa* (Rice A), which corresponds to the PCR fragment of SEQ ID NO:7.

SEQ ID NO:14 - Protein sequence of the rice NIM1 homologue encoded by SEQ ID NO:13.

SEQ ID NO:15 - Partial cDNA sequence of a *NIM1* homologue from *Oryza sativa* (Rice B), which corresponds to the PCR fragment of SEQ ID NO:9.

SEQ ID NO:16 - Protein sequence of the rice NIM1 homologue encoded by SEQ ID NO:15.

SEQ ID NO:17 - Full length cDNA sequence of a *NIM1* homologue from *Triticum aestivum* (Wheat), which corresponds to the PCR fragment of SEQ ID NO:11.

SEQ ID NO:18 - Protein sequence of the wheat NIM1 homologue encoded by SEQ ID NO:17.

SEQ ID NO:19 - Full length cDNA sequence corresponding to the *Triticum aestivum* (wheat) *NIM*-like genomic sequence pHW01 (SEQ ID NO:1).

SEQ ID NO:20 - Protein sequence encoded by SEQ ID NO:19.

In describing the present invention, the following terms may be employed, and are intended to be defined as indicated below.

Associated With / Operatively Linked: Refers to two DNA sequences that are related physically or functionally. For example, a promoter or regulatory DNA sequence is said to be "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

Chimeric Gene: A recombinant DNA sequence in which a promoter or regulatory DNA sequence is operatively linked to, or associated with, a DNA sequence that codes for an mRNA or which is expressed as a protein, such that the regulator DNA sequence is able to regulate transcription or expression of the associated DNA sequence. The regulator DNA sequence of the chimeric gene is not normally operatively linked to the associated DNA sequence as found in nature.

Coding Sequence: a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Preferably the RNA is then translated in an organism to produce a protein.

Complementary: refers to two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between the complementary base residues in the antiparallel nucleotide sequences.

Expression: refers to the transcription and/or translation of an endogenous gene or a transgene in plants. In the case of antisense constructs, for example, expression may refer to the transcription of the antisense DNA only.

Expression Cassette: A nucleic acid sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operatively linked to the nucleotide sequence of interest which is operatively linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host, i.e., the particular nucleic acid sequence of the expression cassette does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide

sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, such as a plant, the promoter can also be specific to a particular tissue, or organ, or stage of development.

Gene: A defined region that is located within a genome and that, besides the aforementioned coding nucleic acid sequence, comprises other, primarily regulatory, nucleic acid sequences responsible for the control of expression, i.e., transcription and translation of the coding portion. A gene may also comprise other 5' and 3' untranslated sequences and termination sequences. Further elements that may be present are, for example, introns.

Heterologous DNA Sequence: The terms "heterologous DNA sequence", "exogenous DNA segment" or "heterologous nucleic acid," as used herein, each refer to a sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified through, for example, the use of DNA shuffling. The terms also includes non-naturally occurring multiple copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is not ordinarily found. Exogenous DNA segments are expressed to yield exogenous polypeptides.

Homologous DNA Sequence: A DNA sequence naturally associated with a host cell into which it is introduced.

Isocoding: A nucleic acid sequence is isocoding with a reference nucleic acid sequence when the nucleic acid sequence encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the reference nucleic acid sequence.

Isolated: In the context of the present invention, an isolated nucleic acid molecule or an isolated enzyme is a nucleic acid molecule or enzyme that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or enzyme may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell.

Minimal Promoter: a promoter element, particularly a TATA element, that is inactive or has greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, a minimal promoter functions to permit transcription.

Native: refers to a gene that is present in the genome of an untransformed cell.

Naturally occurring: the term "naturally occurring" is used to describe an object that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

NIM1: Gene described in Ryals *et al.*, 1997, which is involved in the SAR signal transduction cascade.

NIM1: Protein encoded by the *NIM1* gene

Nucleic acid: the term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof *(e.g.* degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al., Nucleic Acid Res.* 19: 5081 (1991); Ohtsuka *et al., J. Biol. Chem.* 260: 2605-2608 (1985); Rossolini *et al., Mol. Cell. Probes* 8: 91-98 (1994)). The terms "nucleic acid" or "nucleic acid sequence" may also be used interchangeably with gene, cDNA, and mRNA encoded by a gene. In the context of the present invention, the nucleic acid molecule is preferably a segment of DNA. Nucleotides are indicated by their bases by the following standard abbreviations: adenine (A), cytosine (C), thymine (T), and guanine (G).

ORF: Open Reading Frame.

Plant: Any whole plant.

Plant Cell: Structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, a plant organ, or a whole plant.

Plant Cell Culture: Cultures of plant units such as, for example, protoplasts, cell culture cells, cells in plant tissues, pollen, pollen tubes, ovules, embryo sacs, zygotes and embryos at various stages of development.

Plant Material: Refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant.

Plant Organ: A distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower bud, or embryo.

Plant tissue: A group of plant cells organized into a structural and functional unit. Any tissue of a plant *in planta* or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture and any groups of plant cells organized into structural and/or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant tissue.

Promoter: An untranslated DNA sequence upstream of the coding region that contains the binding site for RNA polymerase II and initiates transcription of the DNA. The promoter region may also include other elements that act as regulators of gene expression.

Protoplast: An isolated plant cell without a cell wall or with only parts of the cell wall.

Purified: the term "purified," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein which is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

Recombinant DNA molecule: a combination of DNA molecules that are joined together using recombinant DNA technology

Regulatory Elements: Sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements comprise a promoter operably linked to the nucleotide sequence of interest and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

Selectable marker gene: a gene whose expression in a plant cell gives the cell a selective advantage. The selective advantage possessed by the cells transformed with the selectable marker gene may be due to their ability to grow in the presence of a negative

selective agent, such as an antibiotic or a herbicide, compared to the growth of non-transformed cells. The selective advantage possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or energy source. Selectable marker gene also refers to a gene or a combination of genes whose expression in a plant cell gives the cell both, a negative and a positive selective advantage.

Significant Increase: an increase in enzymatic activity that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater of the activity of the wild-type enzyme in the presence of the inhibitor, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

Substantially identical: the phrase "substantially identical," in the context of two nucleic acid or protein sequences, refers to two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90-95%, and most preferably at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of the coding regions. Furthermore, substantially identical nucleic acid or protein sequences perform substantially the same function.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test

sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally,* Ausubel *et al., infra).*

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al., J. Mol. Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, 1990). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix *(see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89: 10915 (1989)).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences *(see, e.g.,* Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90: 5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.,* total cellular) DNA or RNA. "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic* Acid Probes part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5ºC lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize to its target subsequence, but to no other sequences.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42ºC, with the hybridization being carried out overnight. An example of

highly stringent wash conditions is 0.1 5M NaCl at 72ºC for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65ºC for 15 minutes (*see,* Sambrook, *infra*, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45ºC for 15 minutes. An example low stringency wash for a duplex of, *e.g.,* more than 100 nucleotides, is 4-6x SSC at 40ºC for 15 minutes. For short probes (*e.g.,* about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30ºC. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a reference nucleotide sequence preferably hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

A further indication that two nucleic acid sequences or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with, or specifically binds to, the protein encoded by the second nucleic acid. Thus, a protein is typically substantially identical to a second protein, for example, where the two proteins differ only by conservative substitutions.

The phrase "specifically (or selectively) binds to an antibody," or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding

reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the protein with the amino acid sequence encoded by any of the nucleic acid sequences of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with other proteins except for polymorphic variants. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual,* Cold Spring Harbor Publications, New York "Harlow and Lane"), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acid sequences that encode identical or essentially identical amino acid sequences, or where the nucleic acid sequence does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance the codons CGT, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded protein. Such nucleic acid variations are "silent variations" which are one species of "conservatively modified variations." Every nucleic acid sequence described herein which encodes a protein also describes every possible silent variation, except where otherwise noted. One of skill will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a protein is implicit in each described sequence.

Furthermore, one of skill will recognize that individual substitutions deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids

(typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations," where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following five groups each contain amino acids that are conservative substitutions for one another: Aliphatic: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I); Aromatic: Phenylalanine (F), Tyrosine (Y), Tryptophan (W); Sulfur-containing: Methionine (M), Cysteine (C); Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E), Asparagine (N), Glutamine (Q). *See also,* Creighton (1984) *Proteins, W.H.* Freeman and Company. In addition, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are also "conservatively modified variations."

A "subsequence" refers to a sequence of nucleic acids or amino acids that comprise a part of a longer sequence of nucleic acids or amino acids (e.g., protein) respectively.

Nucleic acids are "elongated" when additional nucleotides (or other analogous molecules) are incorporated into the nucleic acid. Most commonly, this is performed with a polymerase (e.g., a DNA polymerase), e.g., a polymerase which adds sequences at the 3' terminus of the nucleic acid.

Two nucleic acids are "recombined" when sequences from each of the two nucleic acids are combined in a progeny nucleic acid. Two sequences are "directly" recombined when both of the nucleic acids are substrates for recombination. Two sequences are "indirectly recombined" when the sequences are recombined using an intermediate such as a cross-over oligonucleotide. For indirect recombination, no more than one of the sequences is an actual substrate for recombination, and in some cases, neither sequence is a substrate for recombination.

A "specific binding affinity" between two molecules, for example, a ligand and a receptor, means a preferential binding of one molecule for another in a mixture of molecules. The binding of the molecules can be considered specific if the binding affinity is about 1×10^4 M^{-1} to about 1×10^6 M^{-1} or greater.

Transformation: a process for introducing heterologous DNA into a host cell or organism.

"Transformed," "transgenic," and "recombinant" refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid

molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A "non-transformed," "non-transgenic," or "non-recombinant" host refers to a wild-type organism, e.g., a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

The following material has been deposited with the Agricultural Research Service, Patent Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. All restrictions on the availability of the deposited material will be irrevocably removed upon the granting of a patent.

Clone	Accession Number	Date of Deposit
pHW01	NRRL B-30152	July 1, 1999

The present invention concerns monocotyledonous *NIM1* homologues, such as those isolated from *Triticum aestivum* (wheat) and *Oryza sativa* (rice). As described more fully below in the Examples, monocotyledonous *NIM1* homologues according to the invention may be isolated from cDNA and/or genomic DNA libraries by probing with fragments of the tobacco *NIM1* cDNA described in WO 00/53762, the disclosure of which is hereby incorporated by reference in its entirety.

In addition, *NIM1* homologues according to the invention can be isolated from cDNA and/or genomic DNA libraries from monocotyledonous plants by PCR amplification using primers constructed based on the *NIM1* sequences from *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Lycopersicon esculentum*, as well as the *NML* sequences from *Arabidopsis thaliana* (*see*, Example 5: "Design of Degenerate Primers" in WO 00/53762).

Furthermore, monocotyledonous *NIM1* homologues according to the invention can be isolated by PCR using the wheat and rice sequences set forth in the attached sequence listing as the basis for constructing PCR primers. For example, the first and last 20-25 consecutive nucleotides of SEQ ID NO:19 (e.g., nucleotides 1-20 and 1649-1668 of SEQ ID NO:19) can be used as the basis for constructing PCR primers to amplify the cDNA sequence (SEQ ID NO:19) directly from a cDNA library from the source plant (wheat). Other DNA sequences of the invention can likewise be amplified by PCR from cDNA or genomic DNA libraries of monocotyledonous plants using the ends of the DNA sequences set forth in the sequence listing as the basis for PCR primers.

Monocotyledonous *NIM1* homologues, such as the wheat and rice *NIM1* homologues described herein, are predicted to encode proteins involved in the signal transduction cascade responsive to biological and chemical inducers, which leads to systemic acquired resistance in plants. The present invention also concerns the transgenic expression of a monocotyledonous *NIM1* homologue in plants to increase SAR gene expression and enhance disease resistance.

The transgenic expression of a monocotyledonous *NIM1* homologue of the invention in plants is predicted to result in immunity to a wide array of plant pathogens, which include, but are not limited to viruses or viroids, e.g. tobacco or cucumber mosaic virus, ringspot virus or necrosis virus, pelargonium leaf curl virus, red clover mottle virus, tomato bushy stunt virus, and like viruses; fungi, e.g. oomycetes such as *Phythophthora parasitica* and *Peronospora tabacina*; bacteria, e.g. *Pseudomonas syringae* and *Pseudomonas tabaci*; insects such as aphids, e.g. *Myzus persicae*; and lepidoptera, e.g., *Heliothus spp.;* and nematodes, e.g., *Meloidogyne incognita.* The vectors and methods of the invention are useful against a number of disease organisms of maize including but not limited to downy mildews such as *Sclerophthora macrospora*, *Sclerophthora rayissiae*, *Sclerospora graminicola*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari* and *Peronosclerospora maydis*; rusts such as *Puccinia sorphi*, *Puccinia polysora* and *Physopella zeae*; other fungi such as *Cercospora zeae-maydis*, *Colletotrichum graminicola*, *Fusarium monoliforme*, *Gibberella zeae*, *Exserohilum turcicum*, *Kabatiellu zeae*, *Erysiphe graminis*, *Septoria* and *Bipolaris maydis*; and bacteria such as *Erwinia stewartii.*

The methods of the present invention can be utilized to confer disease resistance to a wide variety of plants, including gymnosperms, monocots, and dicots. Although disease resistance can be conferred upon any plants falling within these broad classes, it is

particularly useful in agronomically important crop plants, such as rice, wheat, barley, rye, rape, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

A monocotyledonous *NIM1* homologue coding sequence of the present invention may be inserted into an expression cassette designed for plants to construct a chimeric gene according to the invention using standard genetic engineering techniques. The choice of specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and enhancer appropriate for the achieving the desired pattern and level of expression in the chosen plant host is within the level of skill of the routineer in the art. The resultant molecule, containing the individual elements linked in proper reading frame, may be inserted into a vector capable of being transformed into a host plant cell.

Examples of promoters capable of functioning in plants or plant cells (i.e., those capable of driving expression of associated coding sequences such as those coding for NIM1 homologues in plant cells) include the *Arabidopsis* and maize ubiquitin promoters; cauliflower mosaic virus (CaMV) 19S or 35S promoters and CaMV double promoters; rice actin promoters; PR-1 promoters from tobacco, *Arabidopsis*, or maize; nopaline synthase promoters; small subunit of ribulose bisphosphate carboxylase (ssuRUBISCO) promoters, and the like. Especially preferred is the *Arabidopsis* ubiquitin promoter. The promoters themselves may be modified to manipulate promoter strength to increase expression of the associated coding sequence in accordance with art-recognized procedures. Preferred promoters for use with the present invention are those that confer high level constitutive expression.

Signal or transit peptides may be fused to the monocotyledonous *NIM1* homologue coding sequence in the chimeric DNA constructs of the invention to direct transport of the expressed protein to the desired site of action. Examples of signal peptides include those natively linked to the plant pathogenesis-related proteins, e.g. PR-1, PR-2, and the like. *See, e.g.,* Payne *et al.,* 1988. Examples of transit peptides include the chloroplast transit peptides such as those described in Von Heijne *et al.* (1991), *Mazur et al.* (1987), and Vorst *et al.* (1988); and mitochondrial transit peptides such as those described in Boutry *et al.* (1987). Also included are sequences that result in localization of the encoded protein to

various cellular compartments such as the vacuole. See, for example, Neuhaus *et al.* (1991) and Chrispeels (1991).

The chimeric DNA construct(s) of the invention may contain multiple copies of a promoter or multiple copies of a monocotyledonous *NIM1* homologue coding sequence of the present invention. In addition, the construct(s) may include coding sequences for markers and coding sequences for other peptides such as signal or transit peptides, each in proper reading frame with the other functional elements in the DNA molecule. The preparation of such constructs are within the ordinary level of skill in the art.

Useful markers include peptides providing herbicide, antibiotic or drug resistance, such as, for example, resistance to protoporphyrinogen oxidase inhibitors, hygromycin, kanamycin, G418, gentamycin, lincomycin, methotrexate, glyphosate, phosphinothricin, or the like. These markers can be used to select cells transformed with the chimeric DNA constructs of the invention from untransformed cells. Other useful markers are peptidic enzymes which can be easily detected by a visible reaction, for example a color reaction, for example luciferase, ß-glucuronidase, or ß-galactosidase.

Chimeric genes designed for plant expression such as those described herein can be introduced into the plant cell in a number of art-recognized ways. Those skilled in the art will appreciate that the choice of method might depend on the type of plant (i.e. monocot or dicot) and/or organelle (i.e. nucleus, chloroplast, mitochondria) targeted for transformation. Suitable methods of transforming plant cells include microinjection (Crossway *et al.,* 1986), electroporation (Riggs *et al.,* 1986), *Agrobacterium* mediated transformation (Hinchee *et al.,* 1988; Ishida *et al.,* 1996), direct gene transfer (Paszkowski *et al.,* 1984; Hayashimoto *et al.,* 1990), and ballistic particle acceleration using devices available from Agracetus, Inc., Madison, Wisconsin and Dupont, Inc., Wilmington, Delaware (*see, for example,* U.S. Patent 4,945,050; and McCabe *et al.,* 1988). *See also*, Weissinger *et al.* (1988); Sanford *et al.* (1987) (onion); Christou *et al.* (1988) (soybean); McCabe *et al.* (1988) (soybean); Datta *et al.* (1990) (rice); Klein *et al.* (1988) (maize); Klein *et al.* (1988) (maize); Klein *et al.* (1988) (maize); Fromm *et al.* (1990); and Gordon-Kamm *et al.* (1990) (maize); Svab *et al.* (1990) (tobacco chloroplasts); Gordon-Kamm *et al.* (1993) (maize); Shimamoto *et al.* (1989) (rice); Christou *et al.* (1991) (rice); Datta *et al.* (1990) (rice); European Patent Application EP 0 332 581 (orchardgrass and other *Pooideae*); Vasil *et al.* (1993) (wheat); Weeks *et al.* (1993) (wheat); Wan *et al.* (1994) (barley); Jahne *et al.* (1994) (barley); Umbeck *et al.* (1987) (cotton); Casas *et al.* (1993) (sorghum); Somers *et al.* (1992) (oats); Torbert *et al.* (1995) (oats); Weeks *et al.*,(1993) (wheat); WO 94/13822 (wheat); and Nehra *et al.* (1994) (wheat).

A particularly preferred set of embodiments for the introduction of recombinant DNA molecules into maize by microprojectile bombardment can be found in Koziel *et al.* (1993); Hill *et al.* (1995) and Koziel *et al.* (1996). An additional preferred embodiment is the protoplast transformation method for maize as disclosed in EP 0 292 435.

Once a chimeric gene comprising a monocotyledonous *NIM1* homologue coding sequence has been transformed into a particular plant species, it may be propagated in that species or moved into other varieties of the same species, particularly including commercial varieties, using traditional breeding techniques. Particularly preferred plants of the invention include the agronomically important crops listed above. The genetic properties engineered into the transgenic seeds and plants described above are passed on by sexual reproduction and can thus be maintained and propagated in progeny plants.

EXAMPLES

The invention is illustrated in further detail by the following detailed procedures, preparations, and examples. The examples are for illustration only, and are not to be construed as limiting the scope of the present invention. Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, *et al.*, 1989; by T.J. Silhavy, M.L. Berman, and L.W. Enquist, 1984; and by Ausubel, F.M. *et al.,* 1987.

I. Isolation of Homologues of the *Arabidopsis NIM1* Gene from Monocotyledonous Plants

Example 1: **Isolation of a *NIM1* Homologue from *Triticum aestivum* (Wheat)**

A custom genomic DNA library from *Triticum aestivum* (cv UC703) is constructed in EMBL3 SP6/T7 vectors (Clontech). The library ($1x10^6$ pfu) is screened following the protocol of Clontech Laboratories. Two different fragments of the tobacco *NIM1* cDNA (pNOV1206 - SEQ ID NO:1 of WO 00/53762) are used as probes: the 5'-*NIM1* fragment (nucleotide seq. 1-790; 0.8 kb *Accl*/*Eco*RI fragment isolated from pNOV1206) and the 3'-*NIM1* fragment (nucleotide seq. 1176-1770; 0.6 kb *Kpn*I/*Hind*III fragment isolated from pNOV1206). Plaque lifts, each containing 50,000 clones, a total of $1x\ 10^6$ clones (nitrocellulose membranes, NEN), are made in duplicate from 10 phage plates and each probe is hybridized to 10 membranes. The probe is labeled with P32-dCTP using the method of Prime-ItR II Random Primer Labeling (Stratagene). Hybridization is preferably carried out at 58ºC in hybridization buffer (6xSSPE, 5xDenhards, 0.5% SDS, 100 µg stDNA/ml), and washings are preferably conducted in (I): 2xSSPE, SDS 0.1%, room temperature 10 min, (II): 2xSSPE, SDS 0.1% at 55ºC 15 min, and (III) 1xSSPE, SDS 0.1% at 55ºC 15 min, twice for each washing. A total of nine positive clones are isolated by two additional rounds of plaque purification.

Lambda phage DNA is isolated from K802 lysates according to Zabarovsky and Turina, 1988. Among nine positive candidates, six hybridize to both 3'-*NIM1* and 5'- *NIM1* probes by Southern blotting of restriction digested lambda DNA. Hybridizing DNA fragments are then cloned into pUC19 vector (NEB).

DNA sequence of clone HW01 is determined by primer walking using 18-mers designed on the ABI 3948 DNA Synthesizer. HW01 template is sequenced with Big Dye Terminator Sequencing Reactions, using 400ng template per reaction. Cycle conditions are according to the DT 50-30 Program: 95ºC - 10 sec, 50ºC - 5 sec, 60ºC - 4 min for 29 cycles. Following the thermal cycle condition program, the reactions are precipitated with isopropanol. Samples are loaded onto a polyacrylamide gel and analyzed on the ABI 377 Automated Sequencer.

The HW01 template is also subjected to a Primer Island protocol whereby template is prepared on the Qiagen Robot and sequenced in a 96-well Marsh plate block format. Primers used for the plate sequencing are forward and reverse primers from the Primer Island Kit. Sequencing data is analyzed and assembled using Phred/Phrap and Consed Programs.

One of the subcloned DNA sequences from part of lambda clone #8, named pHW01, bears a 4270 bp *Sac*I insert and is identified as a wheat homologue of the *Arabidopsis NIM1* gene (Ryals *et al.*, 1997). Translated amino acid sequence of the wheat *NIM1* homologue is based on inverted sequence of HW01 (i-HW01) in which the orientation of the *NIM1* homologue is the same as the *Arabidopsis NIM1* sequence. The wheat NIM1 amino acid sequence has 77/68% amino acid similarity/identity to the tobacco NIM1 homologue shown as SEQ ID NO:1 of WO 00/53762, 78/68% to the tomato NIM1 homologue shown as SEQ ID NO:3 of WO 00/53762, 65/51% to *Arabidopsis* NIM1 (Ryals *et al.*, 1997), and 69%, 69%, and 59% nucleotide similarity to the tobacco, tomato, and *Arabidopsis NIM1* genes, respectively (see, Table 1 and Table 2, below).

Table 1. Amino Acid Comparison (Similarity/Identity) of NIM1 Homologues

	Wheat	Tobacco	Tomato	*Arabidopsis*
Wheat	100			
Tobacco	77/68	100		
Tomato	78/68	93/90	100	
Arabidopsis	65/51	65/54	66/55	100

Table 2. Nucleotide Comparison (Identity) of *NIM1* Homologues

	Wheat	Tobacco	Tomato	*Arabidopsis*
Wheat	100			
Tobacco	69	100		
Tomato	69	90	100	
Arabidopsis	59	63	62	100

The genomic sequence of the wheat *NIM1* homologue is shown in SEQ ID NO:1 and the encoded protein sequence is shown in SEQ ID NO:2. The wheat *NIM1* homologue comprising SEQ ID NO:1 was deposited in *E. coli* DH5α as pHW01 with the NRRL (Agricultural Research Service, Patent Culture Collection, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A) on July 1, 1999, and assigned accession no. NRRL B-30152.

Example 2: **PCR Amplification of the Wheat *NIM1* Homologue**

PCR is used to confirm that the wheat *NIM1* homologue originates from the wheat genome. Primers KL1 (19nt, 5'-CCATTGCTACTCTTGCCTC-3' (SEQ ID NO:3)) and KL2 (21nt, 5'-ATCGTTGTCTCCCTTTTAACC-3' (SEQ ID NO:4)) corresponding to nucleotides 1871-1890 and nucleotides 2360-2340, respectively, from the pHW01 subclone sequence are used to prime PCR reactions using wheat UC703 genomic DNA as template. Cycling conditions are 94ºC for 30 sec, 50ºC for 30 sec, and 72ºC for 30 sec, for a total of 35 cycles. A ~500 bp band is obtained and cloned. Sequencing of multiple clones with the correct sized insert reveal that three different sequences are amplified from the wheat genome. All three sequences are highly similar to each other, and one of the sequences aligns precisely with the corresponding region of HW01, indicating the HW01 in fact originates from the wheat genome. A wheat *NIM1* homologue according to the invention can therefore be isolated by PCR from a wheat genomic library using the above-described PCR primers, KL1 and KL2.

Example 3: **Isolation of Monocotyledonous *NIM1* Homologues by Southern Hybridization**

DNA from a monocotyledonous plant is isolated using the miniprep method of Dellaporta *et al.* (1983). Southern blotting is performed according to the standard protocol (Amersharm). The DNA sequence of the wheat *NIM1* homologue corresponding to the *NIM1*-specific "NIM loop" (nucleotides 2180-3251 of i-HW01, a 1.1 kb *Nde*I/*Bgl*II fragment isolated from pHW01) is hybridized to genomic DNA of wheat (cv. UC703) and other monocotyledonous crops (e.g., rice, barley and corn). Hybridization is preferably performed at 65ºC in 5xSSPE, 5xDenhards, 0.5% SDS, 100 µg stDNA/ml, and washing is preferably (I): 2xSSPE, SDS 0.1%, room temperature 10 min, (II): 0.2xSSPE, SDS 0.1% at 65ºC 15 min, and (III) 0.1xSSPE, SDS 0.1% at 65ºC 15 min, twice for each washing. The monocotyledonous crops tested show strong hybridization signals to the wheat *NIM1* sequence, indicating the presence of *NIM1* homologues in these crops. Hybridization signals in wheat genomic DNA indicate that at least four *NIM1* homologues are present in the wheat genome.

The PCR product from wheat genomic DNA that is obtained with PCR primers KL1 and KL2 (SEQ ID NO:3 and SEQ ID NO:4, respectively) is used to probe gel blots of wheat RNA. Hybridization with total RNA reveals one faint transcript. However, hybridization with polyA$^+$ RNA reveals the presence of two transcripts: a smaller, more abundant mRNA transcript and a larger, less abundant mRNA. The smaller transcript corresponds to the size detected in total RNA. Both transcripts appear to be present in equal abundance in RNA isolated from leaf tissue from young wheat plants that are untreated or BTH-treated for 24h. The wheat "NIM loop" described above is also used as a probe.

Example 4: **Isolation of *NIM1* Homologues by PCR from Genomic DNA Libraries of Monocotyledonous Crops**

Primers KL1 and KL2 (SEQ ID NO:3 and SEQ ID NO:4, respectively) are used to clone *NIM1* homologues from other monocotyledonous crops. Using the same cycling conditions as used for wheat genomic DNA amplification (Example 2), bands of approximately 500 bp in size are amplified from rice, corn, and barley genomic DNA libraries. The PCR products from the rice DNA are cloned and sequenced. All sequenced clones are found to contain the same insert, and the sequence of the insert shows strong similarity to the *Arabidopsis NIM1* gene and its crop homologues, indicating that a rice homologue of *NIM1* has been cloned.

Example 5: **Isolation of *NIM1* Homologues by PCR from cDNA Libraries of Monocotyledonous** Crops

Degenerate PCR primers are designed based on conserved regions discovered by using the GCG Seqweb multiple sequence alignment program (Pretty, Wisconsin Genetics Computer Group) to align the *Arabidopsis NIM1* gene (Ryals *et al.,* 1997); the *Arabidopsis thaliana NIM*-like (*NML*) genomic sequences *AtNMLc5, AtNMLc2, AtNMLc4-1*, and *AtNMLc4-2*; and the *NIM1* sequences from *Nicotiana tabacum* and *Lycopersicon esculentum* (See WO 00/53762). Based on this alignment, degenerate PCR primers are designed for PCR amplification of *NIM1* homologues from other crop species including wheat and rice. Two of the primers designed from these conserved regions are listed below in Table 3. Primers are preferably synthesized by Genosys Biotechnologies, Inc. (The Woodlands, Texas). Positions of degeneracy are indicated in Table 3 by the notation of more than one base at a single site in the oligonucleotide. "Orientation" designates whether the primer is directed towards the 3' end (Downstream) or the 5' end (Upstream) of the cDNA.

Table 3: Degenerate Primers

Primer	Sequence (5' to 3')	SEQ ID NO:	Orientation
NIM 2B	GGCACTGGACTCAGATGATGTTGAACT T T T GT	SEQ ID NO:5	Downstream
NIM 2D	AGTTGAGCAAGGCCAACTCGATTTTCAAAAT T C A T GG T	SEQ ID NO:6	Upstream

NIM1 homologue DNA fragments are amplified from wheat and rice using cDNA as template. Degenerate primer PCR is preferably performed with Ready-To-Go PCR Beads (Amersham, Piscataway, NJ) in a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA). 5 to 10 ng of cDNA is used in each reaction, with each primer at a final concentration of 0.8 µM. Preferable cycling parameters are as follows: 94°C for 1 minute; 3 cycles of [94°C for 30 seconds; 37°C for 30 seconds; 72°C for 2 minutes]; 35 cycles of [94°C for 30 seconds; 60°C for 30 seconds; 72°C for 2 minutes]; 72°C for 7 minutes; 4°C hold. Reaction products are analyzed on 2% agarose gels and DNA fragments of the appropriate size are excised. DNA fragments are isolated from agarose bands using, for example, the Geneclean III Kit (BIO 101, Inc., Carlsbad, CA) and cloned using, for example,

the TOPO TA Cloning Kit (Invitrogen Corporation, Carlsbad, CA). Plasmids are isolated using, for example, the CONCERT Rapid Plasmid Miniprep System (Life Technologies, Inc., Rockville, MD) and sequenced by standard protocols.

Using primers 2B and 2D, two unique *NIM1* homologue DNA fragments are amplified from the rice cDNA library (SEQ ID NO:7 and 9) and one unique *NIM1* homologue DNA fragment is amplified from the wheat cDNA library (SEQ ID NO:11).

Example 6: **Full-Length Monocotyledous *NIM1* Homologue cDNA's**

Corresponding cDNA sequences upstream and downstream from the *NIM1* homologue PCR fragments are preferably obtained by RACE PCR using the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA). Preferably, at least three independent RACE products are sequenced for each 5'- or 3'-end in order to eliminate PCR errors. A full-length rice *NIM1* homologue cDNA sequence corresponding to the PCR fragment shown in SEQ ID NO:7 is presented as SEQ ID NO:13; a *NIM1* homologue rice cDNA sequence corresponding to the PCR fragment shown in SEQ ID NO:9 is presented as SEQ ID NO:15; and a full-length wheat *NIM1* homologue cDNA sequence corresponding to the PCR fragment shown in SEQ ID NO:11 is presented as SEQ ID NO:17.

A full-length wheat *NIM1* homologue cDNA sequence corresponding to the wheat *NIM1* genomic sequence pHW01 (SEQ ID NO:1) is preferably obtained by RACE PCR and is presented as SEQ ID NO:19. (The 3' end of SEQ ID NO:19 is from a cDNA prediction program.)

II. Expression of the Gene Sequences of the Invention In Plants

A monocotyledonous *NIM1* homologue of the present invention can be incorporated into plant cells using conventional recombinant DNA technology. Generally, this involves inserting a coding sequence of the invention into an expression system to which the coding sequence is heterologous (i.e., not normally present) using standard cloning procedures known in the art. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences. A large number of vector systems known in the art can be used, such as plasmids, bacteriophage viruses and other modified viruses. Suitable vectors include, but are not limited to, viral vectors such as lambda vector

systems λgtl1, λgtl0 and Charon 4; plasmid vectors such as pBI121, pBR322, pACYC177, pACYC184, pAR series, pKK223-3, pUC8, pUC9, pUC18, pUC19, pLG339, pRK290, pKC37, pKC101, pCDNAII; and other similar systems. The components of the expression system may also be modified to increase expression. For example, truncated sequences, nucleotide substitutions or other modifications may be employed. The expression systems described herein can be used to transform virtually any crop plant cell under suitable conditions. Transformed cells can be regenerated into whole plants such that the monocotyledonous *NIM1* homologue plays a role in increasing SAR gene expression and enhancing disease resistance in the transgenic plants.

Example 7: **Construction of Plant Expression Cassettes**

Coding sequences intended for expression in transgenic plants are first assembled in expression cassettes behind a suitable promoter expressible in plants. The expression cassettes may also comprise any further sequences required or selected for the expression of the transgene. Such sequences include, but are not restricted to, transcription terminators, extraneous sequences to enhance expression such as introns, vital sequences, and sequences intended for the targeting of the gene product to specific organelles and cell compartments. These expression cassettes can then be easily transferred to the plant transformation vectors described below. The following is a description of various components of typical expression cassettes.

1. Promoters

The selection of the promoter used in expression cassettes will determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters will express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and the selection will reflect the desired location of accumulation of the gene product. Alternatively, the selected promoter may drive expression of the gene under various inducing conditions. Promoters vary in their strength, i.e., ability to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters can be used, including the gene's native promoter. The following are non-limiting examples of promoters that may be used in expression cassettes.

a. Constitutive Expression, the Ubiquitin Promoter:

Ubiquitin is a gene product known to accumulate in many cell types and its promoter has been cloned from several species for use in transgenic plants (*e.g.* sunflower - Binet *et al.*,1991; maize - Christensen *et al.*, 1989; and *Arabidopsis* - Norris *et al.*, 1993). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926 (to Lubrizol). Taylor *et al.* (1993) describe a vector (pAHC25) that comprises the maize ubiquitin promoter and first intron and its high activity in cell suspensions of numerous monocotyledons when introduced via microprojectile bombardment. The *Arabidopsis* ubiquitin promoter is especially preferred for use with the *NIM1* homologues of the present invention. The ubiquitin promoter is suitable for gene expression in transgenic plants, both monocotyledons and dicotyledons. Suitable vectors are derivatives of pAHC25 or any of the transformation vectors described in this application, modified by the introduction of the appropriate ubiquitin promoter and/or intron sequences.

b. Constitutive Expression, the CaMV 35S Promoter:

Construction of the plasmid pCGN1761 is described in the published patent application EP 0 392 225 (Example 23). pCGN1761 contains the "double" CaMV 35S promoter and the *tml* transcriptional terminator with a unique *EcoRI* site between the promoter and the terminator and has a pUC-type backbone. A derivative of pCGN1761 is constructed which has a modified polylinker which includes *NotI* and *XhoI* sites in addition to the existing *EcoRI* site. This derivative is designated pCGN1761ENX. pCGN1761ENX is useful for the cloning of cDNA sequences or coding sequences (including microbial ORF sequences) within its polylinker for the purpose of their expression under the control of the 35S promoter in transgenic plants. The entire 35S promoter-coding sequence-*tml* terminator cassette of such a construction can be excised by *HindIII, SphI, SalI,* and *XbaI* sites 5' to the promoter and *XbaI, BamHI* and *BglI* sites 3' to the terminator for transfer to transformation vectors such as those described below. Furthermore, the double 35S promoter fragment can be removed by 5' excision with *HindIII, SphI, SalI, XbaI*, or *PstI*, and 3' excision with any of the polylinker restriction sites (*EcoRI, NotI* or *XhoI*) for replacement with another promoter. If desired, modifications around the cloning sites can be made by the introduction of sequences that may enhance translation. This is particularly useful when overexpression is desired. For example, pCGN1761ENX may be modified by optimization of the translational initiation site as described in Example 37 of U.S. Patent No. 5,639,949.

c. Constitutive Expression, the Actin Promoter:

Several isoforms of actin are known to be expressed in most cell types and consequently the actin promoter is a good choice for a constitutive promoter. In particular, the promoter from the rice *Actl* gene has been cloned and characterized (McElroy *et al.*, 1990). A 1.3kb fragment of the promoter was found to contain all the regulatory elements required for expression in rice protoplasts. Furthermore, numerous expression vectors based on the *Actl* promoter have been constructed specifically for use in monocotyledons (McElroy *et al.*, 1991). These incorporate the *Actl*-intron 1, *Adhl* 5' flanking sequence and *Adhl*-intron 1 (from the maize alcohol dehydrogenase gene) and sequence from the CaMV 35S promoter. Vectors showing highest expression were fusions of 35S and *Actl* intron or the *Actl* 5' flanking sequence and the *Actl* intron. Optimization of sequences around the initiating ATG (of the GUS reporter gene) also enhanced expression. The promoter expression cassettes described by McElroy *et al.* (1991) can be easily modified for gene expression and are particularly suitable for use in monocotyledonous hosts. For example, promoter-containing fragments is removed from the McElroy constructions and used to replace the double 35S promoter in pCGN1761ENX, which is then available for the insertion of specific gene sequences. The fusion genes thus constructed can then be transferred to appropriate transformation vectors. In a separate report, the rice *Actl* promoter with its first intron has also been found to direct high expression in cultured barley cells (Chibbar *et al.*, 1993).

d. Inducible Expression, the PR-1 Promoter:

The double 35S promoter in pCGN1761ENX may be replaced with any other promoter of choice that will result in suitably high expression levels. By way of example, one of the chemically regulatable promoters described in U.S. Patent No. 5,614,395 may replace the double 35S promoter. The promoter of choice is preferably excised from its source by restriction enzymes, but can alternatively be PCR-amplified using primers that carry appropriate terminal restriction sites. Should PCR-amplification be undertaken, then the promoter should be re-sequenced to check for amplification errors after the cloning of the amplified promoter in the target vector. The chemically/pathogen regulatable tobacco PR-1a promoter is cleaved from plasmid pCIB1004 (for construction, see example 21 of EP 0 332 104) and transferred to plasmid pCGN1761ENX (Uknes *et al.*, 1992). pCIB1004 is cleaved with *Ncol* and the resultant 3' overhang of the linearized fragment is rendered

blunt by treatment with T4 DNA polymerase. The fragment is then cleaved with *HindIII* and the resultant PR-1a promoter-containing fragment is gel purified and cloned into pCGN1761ENX from which the double 35S promoter has been removed. This is done by cleavage with *XhoI* and blunting with T4 polymerase, followed by cleavage with *HindIII* and isolation of the larger vector-terminator containing fragment into which the pCIB1004 promoter fragment is cloned. This generates a pCGN1761ENX derivative with the PR-1a promoter and the *tml* terminator and an intervening polylinker with unique *EcoRI* and *NotI* sites. The selected coding sequence can be inserted into this vector, and the fusion products (*i.e.* promoter-gene-terminator) can subsequently be transferred to any selected transformation vector, including those described *infra*. Various chemical regulators may be employed to induce expression of the selected coding sequence in the plants transformed according to the present invention, including the benzothiadiazole, isonicotinic acid, and salicylic acid compounds disclosed in U.S. Patent Nos. 5,523,311 and 5,614,395.

e. Inducible Expression, an Ethanol-Inducible Promoter:

A promoter inducible by certain alcohols or ketones, such as ethanol, may also be used to confer inducible expression of a coding sequence of the present invention. Such a promoter is for example the *alcA* gene promoter from *Aspergillus nidulans* (Caddick *et al.*, 1998). In *A. nidulans*, the *alcA* gene encodes alcohol dehydrogenase I, the expression of which is regulated by the AlcR transcription factors in presence of the chemical inducer. For the purposes of the present invention, the CAT coding sequences in plasmid palcA:CAT comprising a *alcA* gene promoter sequence fused to a minimal 35S promoter (Caddick *et al.*, 1998) are replaced by a coding sequence of the present invention to form an expression cassette having the coding sequence under the control of the *alcA* gene promoter. This is carried out using methods well known in the art.

f. Inducible Expression, a Glucocorticoid-Inducible Promoter:

Induction of expression of a NIM1 homologue of the present invention using systems based on steroid hormones is also contemplated. For example, a glucocorticoid-mediated induction system is used (Aoyama and Chua, 1997) and gene expression is induced by application of a glucocorticoid, for example a synthetic glucocorticoid, preferably dexamethasone, preferably at a concentration ranging from 0.1mM to 1mM, more preferably from 10mM to 100mM. For the purposes of the present invention, the luciferase gene sequences are replaced by a gene sequence encoding a NIM1 homologue to form an

expression cassette having the gene sequence encoding a NIM1 homologue under the control of six copies of the GAL4 upstream activating sequences fused to the 35S minimal promoter. This is carried out using methods well known in the art. The trans-acting factor comprises the GAL4 DNA-binding domain (Keegan *et al.*, 1986) fused to the transactivating domain of the herpes viral protein VP16 (Triezenberg *et al.*, 1988) fused to the hormone-binding domain of the rat glucocorticoid receptor (Picard *et al.*, 1988). The expression of the fusion protein is controlled by any promoter suitable for expression in plants known in the art or described here. This expression cassette is also comprised in the plant comprising the gene sequence encoding a NIM1 homologue fused to the 6xGAL4/minimal promoter. Thus, tissue- or organ-specificity of the fusion protein is achieved leading to inducible tissue- or organ-specificity of the NIM1 homologue.

g. Root Specific Expression:

Another pattern of gene expression is root expression. A suitable root promoter is described by de Framond (1991) and also in the published patent application EP 0 452 269. This promoter is transferred to a suitable vector such as pCGN1761ENX for the insertion of a selected gene and subsequent transfer of the entire promoter-gene-terminator cassette to a transformation vector of interest.

h. Wound-Inducible Promoters:

Wound-inducible promoters may also be suitable for gene expression. Numerous such promoters have been described (*e.g.* Xu *et al.*, 1993); Logemann *et al.*, 1989; Rohrmeier & Lehle, 1993; Firek *et al.*, 1993; Warner *et al.*, 1993) and all are suitable for use with the instant invention. Logemann *et al.* describe the 5' upstream sequences of the dicotyledonous potato *wun1* gene. Xu *et al.* show that a wound-inducible promoter from the dicotyledon potato (*pin2*) is active in the monocotyledon rice. Further, Rohrmeier & Lehle describe the cloning of the maize *Wip1* cDNA which is wound induced and which can be used to isolate the cognate promoter using standard techniques. Similar, Firek *et al.* and Warner *et al.* have described a wound-induced gene from the monocotyledon *Asparagus officinalis,* which is expressed at local wound and pathogen invasion sites. Using cloning techniques well known in the art, these promoters can be transferred to suitable vectors, fused to the genes pertaining to this invention, and used to express these genes at the sites of plant wounding.

i. Pith-Preferred Expression:

Patent Application WO 93/07278 describes the isolation of the maize *trpA* gene, which is preferentially expressed in pith cells. The gene sequence and promoter extending up to -1726 bp from the start of transcription are presented. Using standard molecular biological techniques, this promoter, or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a foreign gene in a pith-preferred manner. In fact, fragments containing the pith-preferred promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

j. Leaf-Specific Expression:

A maize gene encoding phosphoenol carboxylase (PEPC) has been described by Hudspeth & Grula (1989). Using standard molecular biological techniques the promoter for this gene can be used to drive the expression of any gene in a leaf-specific manner in transgenic plants.

k. Pollen-Specific Expression:

WO 93/07278 describes the isolation of the maize calcium-dependent protein kinase (CDPK) gene which is expressed in pollen cells. The gene sequence and promoter extend up to 1400 bp from the start of transcription. Using standard molecular biological techniques, this promoter or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a NIM1 homologue of the present invention in a pollen-specific manner.

2. Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the *tml* terminator, the nopaline synthase terminator and the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons. In addition, a gene's native transcription terminator may be used.

3. Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adh1* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, 1987). In the same experimental system, the intron from the maize *bronze1* gene had a similar effect in enhancing expression. Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (*e.g.* Gallie *et al.*, 1987; Skuzeski *et al.*, 1990).

4. Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence found at the amino terminal end of various proteins which is cleaved during chloroplast import to yield the mature protein (*e.g.* Comai *et al.*, 1988). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck, *et al.*, 1985). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins which are known to be chloroplast localized. *See also*, the section entitled "Expression With Chloroplast Targeting" in Example 37 of U.S. Patent No. 5,639,949.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al.*, 1989). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate

amino transferase isoforms for mitochondria. Targeting cellular protein bodies has been described by Rogers *et al.* (1985).

In addition, sequences have been characterized which cause the targeting of gene products to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, 1990). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.,* 1990).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the amino terminal ATG of the transgene. The signal sequence selected should include the known cleavage site, and the fusion constructed should take into account any amino acids after the cleavage site which are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or, alternatively, replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques described by Bartlett *et al.* (1982) and Wasmann *et al.* (1986). These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes.

The above-described mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell-targeting goal under the transcriptional regulation of a promoter that has an expression pattern different to that of the promoter from which the targeting signal derives.

Example 8: **Construction of Plant Transformation Vectors**

Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation arts, and the genes pertinent to this

invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *nptII* gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra, 1982; Bevan *et al.*, 1983), the *bar* gene, which confers resistance to the herbicide phosphinothricin (White *et al.*, 1990; Spencer *et al.*, 1990), the *hph* gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann), and the *dhfr* gene, which confers resistance to methatrexate (Bourouis *et al.*, 1983), and the EPSPS gene, which confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642).

1. Vectors Suitable for *Agrobacterium* Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens.* These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984)) and pXYZ. Below, the construction of two typical vectors suitable for *Agrobacterium* transformation is described.

a. pCIB200 and pCIB2001:

The binary vectors pcIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and are constructed in the following manner. pTJS75kan is created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, 1985) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Messing & Vierra, 1982; Bevan *et al.*, 1983; McBride *et al.*, 1990). *XhoI* linkers are ligated to the *EcoRV* fragment of PCIB7 which contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein *et al.*, 1987), and the *XhoI*-digested fragment are cloned into *SalI*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI, SstI, KpnI, BglII, XbaI,* and *SalI.* pCIB2001 is a derivative of pCIB200 created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI, SstI, KpnI, BglII, XbaI, SalI, MluI, BclI, AvrII, ApaI, HpaI,* and *StuI.* pCIB2001, in addition to containing these unique restriction sites also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated

transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other hosts, and the *OriT* and *OriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

b. pCIB10 and Hygromycin Selection Derivatives thereof:

The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants and T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium.* Its construction is described by Rothstein *et al.* (1987). Various derivatives of pCIB10 are constructed which incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al.*, 1983). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

2. Vectors Suitable for non-*Agrobacterium* Transformation

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (*e.g.* PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Below, the construction of typical vectors suitable for non-*Agrobacterium* transformation is described.

a. pCIB3064:

pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide basta (or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites are mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *SspI* and *PvuII.* The new restriction sites are 96 and 37 bp away from the unique *SalI* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 is designated pCIB3025. The GUS

gene is then excised from pCIB3025 by digestion with *SalI* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 is obtained from the John Innes Centre, Norwich and the a 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* is excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.*, 1987). This generated pCIB3064, which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI, PstI, HindIII,* and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

b. pSOG19 and pSOG35:

pSOG35 is a transformation vector that utilizes the *E. coli* gene dihydrofolate reductase (DFR) as a selectable marker conferring resistance to methotrexate. PCR is used to amplify the 35S promoter (-800 bp), intron 6 from the maize Adh1 gene (-550 bp) and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250-bp fragment encoding the *E. coli* dihydrofolate reductase type II gene is also amplified by PCR and these two PCR fragments are assembled with a *SacI-PstI* fragment from pB1221 (Clontech) which comprises the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generates pSOG19 which contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generates the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and have *HindIII, SphI, PstI* and *EcoRI* sites available for the cloning of foreign substances.

Example 9: **Transformation**

Once the gene sequence of interest has been cloned into an expression system, it is transformed into a plant cell. Methods for transformation and regeneration of plants are well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, micro-injection, and microprojectiles. In addition, bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Below are descriptions of representative techniques for transforming both dicotyledonous and monocotyledonous plants.

1. Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski *et al.*, 1984; Potrykus *et al.*, 1985; Reich *et al.*, 1986; and Klein *et al.*, 1987. In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. *Agrobacterium* transformation typically involves the transfer of the binary vector carrying the foreign DNA of interest (*e.g.* pCIB200 or pCIB2001) to an appropriate *Agrobacterium* strain which may depend of the complement of *vir* genes carried by the host *Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (*e.g.* strain CIB542 for pCIB200 and pCIB2001 (Uknes *et al.*, 1993). The transfer of the recombinant binary vector to *Agrobacterium* is accomplished by a triparental mating procedure using *E. coli* carrying the recombinant binary vector, a helper *E. coli* strain which carries a plasmid such as pRK2013 and which is able to mobilize the recombinant binary vector to the target *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to *Agrobacterium* by DNA transformation (Höfgen & Willmitzer, 1988).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or biologically active particles at plant tissues and cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the desired gene. Alternatively, the target cell can be

surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried yeast cells, dried bacterium or a bacteriophage, each containing DNA sought to be introduced) can also be propelled into plant cell tissue.

2. Transformation of Monocotyledons

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, and particle bombardment into callus tissue. Transformations can be undertaken with a single DNA species or multiple DNA species (*i.e.* co-transformation) and both these techniques are suitable for use with this invention. Co-transformation may have the advantage of avoiding complete vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded desirable. However, a disadvantage of the use of co-transformation is the less than 100% frequency with which separate DNA species are integrated into the genome (Schocher *et al.*, 1986).

Patent Applications EP 0 292 435, EP 0 392 225, and WO 93/07278 describe techniques for the preparation of callus and protoplasts from an elite inbred line of maize, transformation of protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm *et al.* (1990) and Fromm *et al.* (1990) have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, WO 93/07278 and Koziel *et al.* (1993) describe techniques for the transformation of elite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for bombardment.

Transformation of rice can also be undertaken by direct gene transfer techniques utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been described for *Japonica*-types and *Indica*-types (Zhang *et al.*, 1988; Shimamoto *et al.*, 1989; Datta *et al.*, 1990). Both types are also routinely transformable using particle bombardment (Christou *et al.*, 1991). Furthermore, WO 93/21335 describes techniques for the transformation of rice via electroporation.

Patent Application EP 0 332 581 describes techniques for the generation, transformation and regeneration of Pooideae protoplasts. These techniques allow the

transformation of *Dactylis* and wheat. Furthermore, wheat transformation has been described by Vasil *et al.* (1992) using particle bombardment into cells of type C long-term regenerable callus, and also by Vasil *et al.* (1993) and Weeks *et al.* (1993) using particle bombardment of immature embryos and immature embryo-derived callus. A preferred technique for wheat transformation, however, involves the transformation of wheat by particle bombardment of immature embryos and includes either a high sucrose or a high maltose step prior to gene delivery. Prior to bombardment, any number of embryos (0.75-1 mm in length) are plated onto MS medium with 3% sucrose (Murashiga & Skoog, 1962) and 3 mg/l 2,4-D for induction of somatic embryos, which is allowed to proceed in the dark. On the chosen day of bombardment, embryos are removed from the induction medium and placed onto the osmoticum (*i.e.* induction medium with sucrose or maltose added at the desired concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 h and are then bombarded. Twenty embryos per target plate is typical, although not critical. An appropriate gene-carrying plasmid (such as pCIB3064 or pSG35) is precipitated onto micrometer size gold particles using standard procedures. Each plate of embryos is shot with the DuPont Biolistics® helium device using a burst pressure of ~1000 psi using a standard 80 mesh screen. After bombardment, the embryos are placed back into the dark to recover for about 24 h (still on osmoticum). After 24 hrs, the embryos are removed from the osmoticum and placed back onto induction medium where they stay for about a month before regeneration. Approximately one month later the embryo explants with developing embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter GA), further containing the appropriate selection agent (10 mg/l basta in the case of pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one month, developed shoots are transferred to larger sterile containers known as "GA7s" which contain half-strength MS, 2% sucrose, and the same concentration of selection agent.

Tranformation of monocotyledons using *Agrobacterium* has also been described. *See,* WO 94/00977 and U.S. Patent No. 5,591,616.

III. Breeding and Seed Production

Example 10: **Breeding**

The plants obtained via tranformation with a gene of the present invention can be any of a wide variety of plant species, including those of monocots and dicots; however, the plants used in the method of the invention are preferably selected from the list of agronomically important target crops set forth *supra*. The expression of a gene of the present invention in combination with other characteristics important for production and quality can be incorporated into plant lines through breeding. Breeding approaches and techniques are known in the art. See, for example, Welsh J. R. (1981); Wood D. R. (Ed.) (1983); Mayo O. (1987); Singh, D.P. (1986); and Wricke and Weber (1986).

The genetic properties engineered into the transgenic seeds and plants described above are passed on by sexual reproduction or vegetative growth and can thus be maintained and propagated in progeny plants. Generally said maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as tilling, sowing or harvesting. Specialized processes such as hydroponics or greenhouse technologies can also be applied. As the growing crop is vulnerable to attack and damages caused by insects or infections as well as to competition by weed plants, measures are undertaken to control weeds, plant diseases, insects, nematodes, and other adverse conditions to improve yield. These include mechanical measures such a tillage of the soil or removal of weeds and infected plants, as well as the application of agrochemicals such as herbicides, fungicides, gametocides, nematicides, growth regulants, ripening agents and insecticides.

Use of the advantageous genetic properties of the transgenic plants and seeds according to the invention can further be made in plant breeding, which aims at the development of plants with improved properties such as tolerance of pests, herbicides, or stress, improved nutritional value, increased yield, or improved structure causing less loss from lodging or shattering. The various breeding steps are characterized by well-defined human intervention such as selecting the lines to be crossed, directing pollination of the parental lines, or selecting appropriate progeny plants. Depending on the desired properties, different breeding measures are taken. The relevant techniques are well known in the art and include but are not limited to hybridization, inbreeding, backcross breeding, multiline breeding, variety blend, interspecific hybridization, aneuploid techniques, etc. Hybridization techniques also include the sterilization of plants to yield male or female sterile plants by mechanical, chemical, or biochemical means. Cross pollination of a male sterile plant with pollen of a different line assures that the genome of the male sterile but female fertile plant will uniformly obtain properties of both parental lines. Thus, the

transgenic seeds and plants according to the invention can be used for the breeding of improved plant lines, that for example, increase the effectiveness of conventional methods such as herbicide or pestidice treatment or allow one to dispense with said methods due to their modified genetic properties. Alternatively new crops with improved stress tolerance can be obtained, which, due to their optimized genetic "equipment", yield harvested product of better quality than products that were not able to tolerate comparable adverse developmental conditions.

Example 11: **Seed Production**

In seeds production, germination quality and uniformity of seeds are essential product characteristics, whereas germination quality and uniformity of seeds harvested and sold by the farmer is not important. As it is difficult to keep a crop free from other crop and weed seeds, to control seedborne diseases, and to produce seed with good germination, fairly extensive and well-defined seed production practices have been developed by seed producers, who are experienced in the art of growing, conditioning and marketing of pure seed. Thus, it is common practice for the farmer to buy certified seed meeting specific quality standards instead of using seed harvested from his own crop. Propagation material to be used as seeds is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides, or mixtures thereof. Customarily used protectant coatings comprise compounds such as captan, carboxin, thiram (TMTD®), methalaxyl (Apron®), and pirimiphos-methyl (Actellic®). If desired, these compounds are formulated together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal or animal pests. The protectant coatings may be applied by impregnating propagation material with a liquid formulation or by coating with a combined wet or dry formulation. Other methods of application are also possible such as treatment directed at the buds or the fruit.

It is a further aspect of the present invention to provide new agricultural methods, such as the methods examplified above, which are characterized by the use of transgenic plants, transgenic plant material, or transgenic seed according to the present invention.

The seeds may be provided in a bag, container or vessel comprised of a suitable packaging material, the bag or container capable of being closed to contain seeds. The bag, container or vessel may be designed for either short term or long term storage, or both,

of the seed. Examples of a suitable packaging material include paper, such as kraft paper, rigid or pliable plastic or other polymeric material, glass or metal. Desirably the bag, container, or vessel is comprised of a plurality of layers of packaging materials, of the same or differing type. In one embodiment the bag, container or vessel is provided so as to exclude or limit water and moisture from contacting the seed. In one example, the bag, container or vessel is sealed, for example heat sealed, to prevent water or moisture from entering. In another embodiment water absorbent materials are placed between or adjacent to packaging material layers. In yet another embodiment the bag, container or vessel, or packaging material of which it is comprised is treated to limit, suppress or prevent disease, contamination or other adverse affects of storage or transport of the seed. An example of such treatment is sterilization, for example by chemical means or by exposure to radiation. Comprised by the present invention is a commercial bag comprising seed of a transgenic plant comprising a gene of the present invention that is expressed in said transformed plant at higher levels than in a wild type plant, together with a suitable carrier, together with label instructions for the use thereof for conferring broad spectrum disease resistance to plants.

IV. Disease Resistance Evaluation

Disease resistance evaluation is performed by methods known in the art. *See*, Uknes *et al.* (1993); Görlach *et al.* (1996); Alexander *et al.* (1993). For example, several representative disease resistance assays are described below.

Example 12: ***Phytophthora parasitica* (Black Shank) Resistance Assay**

Assays for resistance to *Phytophthora parasitica*, the causative organism of black shank, are performed on six-week-old plants grown as described in Alexander *et al.* (1993). Plants are watered, allowed to drain well, and then inoculated by applying 10 ml of a sporangium suspension (300 sporangia/ml) to the soil. Inoculated plants are kept in a greenhouse maintained at 23-25°C day temperature, and 20-22°C night temperature. The wilt index used for the assay is as follows: 0=no symptoms; 1=no symptoms; 1=some sign of wilting, with reduced turgidity; 2=clear wilting symptoms, but no rotting or stunting; 3=clear wilting symptoms with stunting, but no apparent stem rot; 4=severe wilting, with visible stem rot and some damage to root system; 5=as for 4, but plants near death or

dead, and with severe reduction of root system. All assays are scored blind on plants arrayed in a random design.

Example 13: ***Pseudomonas syringae* Resistance Assay**

Pseudomonas syringae pv. tabaci strain #551 is injected into the two lower leaves of several 6-7-week-old plants at a concentration of 10^6 or 3 x 10^6 per ml in H_20. Six individual plants are evaluated at each time point. *Pseudomonas tabaci* infected plants are rated on a 5 point disease severity scale, 5=100% dead tissue, 0=no symptoms. A T-test (LSD) is conducted on the evaluations for each day and the groupings are indicated after the Mean disease rating value. Values followed by the same letter on that day of evaluation are not statistically significantly different.

Example 14: ***Cercospora nicotianae* Resistance Assay**

A spore suspension of *Cercospora nicotianae* (ATCC #18366) (100,000-150,000 spores per ml) is sprayed to imminent run-off onto the surface of the leaves. The plants are maintained in 100% humidity for five days. Thereafter the plants are misted with water 5-10 times per day. Six individual plants are evaluated at each time point. *Cercospora nicotianae* is rated on a % leaf area showing disease symptoms basis. A T-test (LSD) is conducted on the evaluations for each day and the groupings are indicated after the Mean disease rating value. Values followed by the same letter on that day of evaluation are not statistically significantly different.

Example 15: ***Peronospora parasitica* Resistance Assay**

Assays for resistance to *Peronospora parasitica* are performed on plants as described in Uknes *et al*, (1993). Plants are inoculated with a compatible isolate of *P. parasitica* by spraying with a conidial suspension (approximately 5 x 10^4 spores per milliliter). Inoculated plants are incubated under humid conditions at 17° C in a growth chamber with a 14-hr day/10-hr night cycle. Plants are examined at 3-14 days, preferably 7-12 days, after inoculation for the presence of conidiophores. In addition, several plants from each treatment are randomly selected and stained with lactophenol-trypan blue (Keogh *et al.*, 1980) for microscopic examination.

The above disclosed embodiments are illustrative. This disclosure of the invention will place one skilled in the art in possession of many variations of the invention. All such obvious and foreseeable variations are intended to be encompassed by the claims.

Applicant's or agent's file reference	A -31281A	International application No

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 18, line 9-16

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Agricultural Research Service, Patent Culture Collection (NRRL)
International Depositary Authority

Address of depositary institution *(including postal code and country)*
1815 North University Street
Peoria, Illinois 61604
United States of America (U.S.A)

Date of deposit	Accession Number
01 July 1999 (01.07.99)	NRRL B-30152

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

We request the Expert Solution where available.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer NATHALIE KUIPER	Authorized officer

Form PCT/RO/134 (July1998)

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence from a monocotyledonous plant that is a homologue of the *NIM1* gene.

2 An isolated nucleic acid molecule according to claim 1, comprising:
   (a) a nucleotide sequence that encodes SEQ ID NO:2, 8, 10, 12, 14, 16, 18, or 20;
   (b) SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19;
   (c) a nucleotide sequence that comprises an at least 20 consecutive base pair portion identical in sequence to an at least 20 consecutive base pair portion of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19;
   (d) a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6;
   (e) a nucleotide sequence that can be amplified from a *Orzya sativa* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6;
   (f) a nucleotide sequence that can be amplified from a *Triticum aestivum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6;
   (g) a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with a pair of primers comprising the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19; or
   (h) a nucleotide sequence that hybridizes to the complement of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19 under stringent hybridization and wash conditions.

3. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that encodes SEQ ID NO:2, 8, 10, 12, 14, 16, 18, or 20.

4. An isolated nucleic acid molecule according to claim 2, comprising SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

5. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that comprises an at least 20 consecutive base pair portion identical in sequence to an at least 20 consecutive base pair portion of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

6. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

7. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that can be amplified from a *Orzya sativa* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

8. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that can be amplified from a *Triticum aestivum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

9. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that can be amplified from a monocotyledonous plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19.

10. An isolated nucleic acid molecule according to claim 2, comprising a nucleotide sequence that hybridizes to the complement of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19 under stringent hybridization and wash conditions.

11. A chimeric gene comprising a promoter active in plants operatively linked to the nucleic acid molecule of claim 1.

12. A recombinant vector comprising the chimeric gene of claim 11.

13. A host cell comprising the chimeric gene of claim 11.

14. A plant comprising the chimeric gene of claim 13.

15. The plant of claim 14, which is a monocotyledonous plant.

16. The plant of claim 14, which is selected from the following: rice, wheat, barley, rye, corn, potato, canola, sunflower, carrot, sweet potato, sugarbeet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry,

pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

17. Seed from the plant of claim 14.

18. A method of increasing SAR gene expression in a plant, comprising expressing the chimeric gene of claim 11 in said plant.

19. A method of enhancing disease resistance in a plant, comprising expressing the chimeric gene of claim 11 in said plant.

20. A PCR primer that is SEQ ID NO:3 or SEQ ID NO:4.

21. A method for isolating a *NIM1* homologue involved in the signal transduction cascade leading to systemic acquired resistance in plants comprising amplifying a DNA molecule from a plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, or 19 or with the pair of primers set forth as SEQ ID NO:3 and 4 or SEQ ID NO:5 and 6.

22. The method of claim 21, wherein said plant DNA library is a *Oryza sativa* (rice) or *Triticum aestivum* (wheat) DNA library.

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aagaaagaaa gaaaaatatc aataggatta ctggtgtaga taaggttgaa atcccagatt 2752

ttattctctc tcctgttaag aactgaagag tgaacccaaa tatgtacaca tacgaaatgt 2812

taattggtgg tcgaattgct aatgattact ctcgcatgtg tgtgcgcgtg cattcagcta 2872

taagatgaca ttttaccaag cttatgtatc tggactaact gcaatctgaa tgtttatacc 2932

a gtt gaa ctc ggc aaa cgt ttc ttc cca cgc tgt tca aat gtg ctg gac 2981

aag atc atg gac gat gaa cct gag ctg gct tcc ctc gga aga gat gca 3029

tcc tcc gag agg aag agg agg ttt cac gac ctg caa gat acg ctt ctg 3077

aag gcg ttc agc gag gac aag gag gag ttt aac aga acg aca acc ctt 3125

tca tct tcg tca tcg tcg acg tcc act gta gca agg aac ttg gca ggt 3173

cga act agg aga tga gcaccctggc ccattcttgc catattgata gctgattctt 3228

tttccgtctg aaactgctcg ctagatcttt cttttctatt taacgagtac tattagtgta 3288

gcatcatcgt cagatatgat gaagctgttg gctttggccc tgtaaatcgc ctagttatgc 3348

tcatttttgc ttgtatagta accgttctcc atacagtaat ttgttgatgg agcttatgga 3408

tccatgtagt agcagccctt ggcctctgac tttgatgatt ggtgagaaac agatggtaga 3468

acattaaaaa aaaacagatg gtagtttctg ctttgcttga ttgatctgca tgactgaata 3528

ctagcactgc ccctgtagtt tatcactgcc cctgtagttt atctctgaag catctaccgc 3588

tgaaaaggac tactctgttt ttaccttgtt acagtcgcca ttttgctgcg ccttctgtcc 3648

taccagtagt attgccgaag tgaaatttat cttgctggac tgaactaccg aggcagagca 3708

aattcaccac caaaaaccat ggagtaaatt acatccacgg tacgctaggg ttctcacttc 3768

gtcaccgaaa gtgtcgaagt tcaccaattt aagttgtcaa aatatctcac tctaagatca 3828

gcattgactt cggaacgcag gcagtagtgg tcctcgccgc cggaacatcg ggggtccgca 3888

cgagggcgcc tgtcctcgac gttccgcgtc cagtggaagt gctcgacgag cggccactcc 3948

gtggcgctgg ctggccgcgt ccagtggaag ttccgtgccc gtgaccggtg accgtgatgc 4008

gccggtgacc gtgggcagcc agcgtccaga gcagcagcga gagggccacg ccgcgtcagg 4068

acgccactcc cgcgtctata atatcagaca cacatacttg acaatcattt caaatcccca 4128

aattatgaat tttgattcaa aatttagatt caaattcaaa cataacaaat ggtccaaatt 4188

aaaccattcg aagtaaaaca caatacatag aaatttattg gtcatgaagc gaccacaaat 4248

gctcagctag atcctcgagc tc 4270

<210> 2
<211> 405
<212> PRT
<213> Triticum aestivum

<400> 2

Met Pro Phe Phe Ser Met Gln Arg His Leu Leu Asp Phe Leu Asp Lys
1 5 10 15

Val Glu Val Asp Asn Leu Pro Leu Ile Leu Ser Val Ala Asn Leu Cys
20 25 30

Asn Lys Ser Cys Val Lys Leu Phe Glu Arg Cys Met Glu Met Val Val
35 40 45

Arg Ser Asn Leu Asp Met Ile Thr Leu Glu Lys Ala Leu Pro Gln Asp
50 55 60

Val Ile Lys Gln Ile Thr Asp Leu Arg Ile Thr Leu Gly Leu Ala Ser
65 70 75 80

Pro Glu Asp Asn Gly Phe Pro Asn Lys His Val Arg Arg Ile Leu Arg
85 90 95

```
Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu Thr Glu
            100                 105                 110

Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala Val Glu
        115                 120                 125

His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Ile Ala Leu Ala
    130                 135                 140

Asp Val Asn Leu Arg Asn Pro Arg Gly Tyr Thr Val Leu His Ile Ala
145                 150                 155                 160

Ala Lys Arg Arg Asp Pro Lys Ile Val Val Ser Leu Leu Thr Lys Gly
                165                 170                 175

Ala Arg Pro Ser Asp Phe Thr Phe Asp Gly Arg Lys Ala Val Gln Ile
            180                 185                 190

Ser Lys Arg Leu Thr Lys His Gly Asp Tyr Phe Gly Asn Thr Glu Glu
        195                 200                 205

Gly Lys Pro Ser Pro Asn Asp Lys Leu Cys Ile Glu Ile Leu Glu Gln
    210                 215                 220

Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Leu Ser Leu Ala
225                 230                 235                 240

Leu Ala Gly Asp Cys Leu Arg Gly Lys Leu Leu Tyr Leu Glu Asn Arg
                245                 250                 255

Val Ala Leu Ala Arg Ile Met Phe Pro Ile Glu Ala Arg Val Ala Met
            260                 265                 270

Asp Ile Ala Gln Val Asp Gly Thr Leu Glu Phe Thr Leu Gly Ser Ser
        275                 280                 285

Thr Asn Pro Pro Leu Glu Ile Thr Thr Val Asp Leu Asn Asp Thr Ser
    290                 295                 300

Phe Lys Met Lys Glu Glu His Leu Ala Arg Met Arg Ala Leu Ser Lys
305                 310                 315                 320

Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Asn Val Leu
                325                 330                 335

Asp Lys Ile Met Asp Asp Glu Pro Glu Leu Ala Ser Leu Gly Arg Asp
            340                 345                 350

Ala Ser Ser Glu Arg Lys Arg Arg Phe His Asp Leu Gln Asp Thr Leu
        355                 360                 365

Leu Lys Ala Phe Ser Glu Asp Lys Glu Glu Phe Asn Arg Thr Thr Thr
    370                 375                 380

Leu Ser Ser Ser Ser Ser Ser Thr Ser Thr Val Ala Arg Asn Leu Ala
385                 390                 395                 400
```

Gly Arg Thr Arg Arg
405

<210> 3
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer KL1

<400> 3
ccattgctac tcttgcctc 19

<210> 4
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer KL2

<400> 4
atcgttgtct ccctttaac c 21

<210> 5
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer NIM 2B

<400> 5
ggcaytggay tcwgatgatg ttgaryt 27

<210> 6
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer NIM 2D

<400> 6
agttkagcma gdccaactck attttcaarr t 31

<210> 7

<211> 498
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (2)..(496)

<400> 7

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g gca ytg gat tct gat gat gtt gag ctt gtg aag ttg ctt ctt aac gaa 49
  Ala Xaa Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu
    1               5                  10                  15

tct gag atc acc ttg gat gat gcc aat gca ttg cac tat gct gct gct   97
Ser Glu Ile Thr Leu Asp Asp Ala Asn Ala Leu His Tyr Ala Ala Ala
             20                  25                  30

tac tgt gat tcg aaa gtt gtt tcg gag ttg tta gac ttg aga ctt gcc   145
Tyr Cys Asp Ser Lys Val Val Ser Glu Leu Leu Asp Leu Arg Leu Ala
         35                  40                  45

aac ttg aat ttg aag aat tcg cgt gga tac acg gca ctc cat ctg gct   193
Asn Leu Asn Leu Lys Asn Ser Arg Gly Tyr Thr Ala Leu His Leu Ala
     50                  55                  60

gct atg agg aga gag cca gct att atc atg tgt ctc cta aac aaa gga   241
Ala Met Arg Arg Glu Pro Ala Ile Ile Met Cys Leu Leu Asn Lys Gly
 65                  70                  75                  80

gca gct gta tca caa ttg act gct gat ggc cag agt gca atg agt atc   289
Ala Ala Val Ser Gln Leu Thr Ala Asp Gly Gln Ser Ala Met Ser Ile
                 85                  90                  95

tgc cgg agg tta aca agg atg aaa gac tac aat aca aag atg gag caa   337
Cys Arg Arg Leu Thr Arg Met Lys Asp Tyr Asn Thr Lys Met Glu Gln
            100                 105                 110

ggc caa gag tca aac aaa gac aga tta tgt att gat ata tta gat agg   385
Gly Gln Glu Ser Asn Lys Asp Arg Leu Cys Ile Asp Ile Leu Asp Arg
        115                 120                 125

gag atg ata agg aaa cct atg gca gtg gaa gat tct gtc acc tcg cct   433
Glu Met Ile Arg Lys Pro Met Ala Val Glu Asp Ser Val Thr Ser Pro
    130                 135                 140

ttg ttg gct gac gat ctt cac atg aag ctt ctc tac ctt gaa aat cga   481
Leu Leu Ala Asp Asp Leu His Met Lys Leu Leu Tyr Leu Glu Asn Arg
145                 150                 155                 160

gtt ggc ctt gct aaa ct                                           498
Val Gly Leu Ala Lys
                165
```

<210> 8
<211> 165

<212> PRT
<213> Oryza sativa

<400> 8
Ala Xaa Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu
1 5 10 15

Ser Glu Ile Thr Leu Asp Asp Ala Asn Ala Leu His Tyr Ala Ala Ala
20 25 30

Tyr Cys Asp Ser Lys Val Val Ser Glu Leu Leu Asp Leu Arg Leu Ala
35 40 45

Asn Leu Asn Leu Lys Asn Ser Arg Gly Tyr Thr Ala Leu His Leu Ala
50 55 60

Ala Met Arg Arg Glu Pro Ala Ile Ile Met Cys Leu Leu Asn Lys Gly
65 70 75 80

Ala Ala Val Ser Gln Leu Thr Ala Asp Gly Gln Ser Ala Met Ser Ile
85 90 95

Cys Arg Arg Leu Thr Arg Met Lys Asp Tyr Asn Thr Lys Met Glu Gln
100 105 110

Gly Gln Glu Ser Asn Lys Asp Arg Leu Cys Ile Asp Ile Leu Asp Arg
115 120 125

Glu Met Ile Arg Lys Pro Met Ala Val Glu Asp Ser Val Thr Ser Pro
130 135 140

Leu Leu Ala Asp Asp Leu His Met Lys Leu Leu Tyr Leu Glu Asn Arg
145 150 155 160

Val Gly Leu Ala Lys
165

<210> 9
<211> 498
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (2)..(496)

<400> 9
g gca ttg gat tca gat gat gtt gag tta gtc agg atg ctg ctc act gaa 49
Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu Thr Glu
1 5 10 15

gga cag aca aat ctt gat gat gcg ttt gca ctg cac tac gcc gtc gaa 97
Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala Val Glu
20 25 30

```
cat tgt gac tcc aaa att aca acc gag ctt ttg gat ctc gca ctt gca   145
His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Leu Ala Leu Ala
         35                  40                  45

gat gtt aat cat aga aac cca aga ggt tat acc gtt ctt cac att gct   193
Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Ile Ala
     50                  55                  60

gcg agg cga aga gag cct aaa atc att gtc tcc ctt tta acc aag ggg   241
Ala Arg Arg Arg Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly
 65                  70                  75                  80

gct cgg cca gca gat gtt aca ttc gat ggg aga aaa gcg gtt caa atc   289
Ala Arg Pro Ala Asp Val Thr Phe Asp Gly Arg Lys Ala Val Gln Ile
                 85                  90                  95

tca aaa aga cta aca aaa caa ggg gat tac ttt ggg gtt acc gaa gaa   337
Ser Lys Arg Leu Thr Lys Gln Gly Asp Tyr Phe Gly Val Thr Glu Glu
            100                 105                 110

gga aaa cct tct cca aaa gat agg tta tgt att gaa ata ctg gag caa   385
Gly Lys Pro Ser Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln
        115                 120                 125

gct gaa aga agg gac cca caa ctc gga gaa gca tca gtt tct ctt gca   433
Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Val Ser Leu Ala
    130                 135                 140

atg gca ggt gag agt cta cga gga agg ttg ctg tac ctt gaa aat cga   481
Met Ala Gly Glu Ser Leu Arg Gly Arg Leu Leu Tyr Leu Glu Asn Arg
145                 150                 155                 160

gtt ggc ctg gct caa ct                                           498
Val Gly Leu Ala Gln
                165
```

<210> 10
<211> 165
<212> PRT
<213> Oryza sativa

<400> 10

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Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu Thr Glu
  1               5                  10                  15

Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala Val Glu
             20                  25                  30

His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Leu Ala Leu Ala
         35                  40                  45

Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Ile Ala
     50                  55                  60
```

```
Ala Arg Arg Arg Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly
 65                  70                  75                  80

Ala Arg Pro Ala Asp Val Thr Phe Asp Gly Arg Lys Ala Val Gln Ile
                 85                  90                  95

Ser Lys Arg Leu Thr Lys Gln Gly Asp Tyr Phe Gly Val Thr Glu Glu
            100                 105                 110

Gly Lys Pro Ser Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln
        115                 120                 125

Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Val Ser Leu Ala
    130                 135                 140

Met Ala Gly Glu Ser Leu Arg Gly Arg Leu Leu Tyr Leu Glu Asn Arg
145                 150                 155                 160

Val Gly Leu Ala Gln
                165
```

<210> 11
<211> 498
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (2)..(496)

<400> 11

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g gca ctg gat tca gat gat gtt gag ctt gtg aag ttg ctt ctt aat gag 49
  Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu
    1               5                  10                  15

tct gaa atc acc cta gac gac gcc aac gca ttg cat tat gct gca gct   97
Ser Glu Ile Thr Leu Asp Asp Ala Asn Ala Leu His Tyr Ala Ala Ala
            20                  25                  30

tac tgc gat tct aaa gtt ctt aca gag ttg tta ggc ctg gaa ctt gcc   145
Tyr Cys Asp Ser Lys Val Leu Thr Glu Leu Leu Gly Leu Glu Leu Ala
         35                  40                  45

aac ttg aat ttg aag aac agt cgt ggg tac aca gca ctc cac cta gct   193
Asn Leu Asn Leu Lys Asn Ser Arg Gly Tyr Thr Ala Leu His Leu Ala
     50                  55                  60

gct atg agg aga gaa cca gct att att atg tgt ctc tta agc aaa gga   241
Ala Met Arg Arg Glu Pro Ala Ile Ile Met Cys Leu Leu Ser Lys Gly
 65                  70                  75                  80

gca gtg gcg tcg caa ttg aca gat gat ggc cgc ctt gca agt aat att   289
Ala Val Ala Ser Gln Leu Thr Asp Asp Gly Arg Leu Ala Ser Asn Ile
                 85                  90                  95
```

```
tgt cga aga tta aca aga cta aaa gat tac aat gca aag atg gag cag   337
Cys Arg Arg Leu Thr Arg Leu Lys Asp Tyr Asn Ala Lys Met Glu Gln
            100                 105                 110

ggc caa gag tca aat aaa gat agg atg tgc att gac atc cta gag agg   385
Gly Gln Glu Ser Asn Lys Asp Arg Met Cys Ile Asp Ile Leu Glu Arg
        115                 120                 125

gag atg atg agg aat cct atg aca gcg gaa gat tca gtc acc tca cct   433
Glu Met Met Arg Asn Pro Met Thr Ala Glu Asp Ser Val Thr Ser Pro
    130                 135                 140

tta ttg gct gat gat ctt cac atg aaa cta agc tac ctt gaa aat cga   481
Leu Leu Ala Asp Asp Leu His Met Lys Leu Ser Tyr Leu Glu Asn Arg
145                 150                 155                 160

gtt ggc ctt gct caa ct                                           498
Val Gly Leu Ala Gln
                165
```

<210> 12
<211> 165
<212> PRT
<213> Triticum aestivum

<400> 12

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Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu
  1               5                  10                  15

Ser Glu Ile Thr Leu Asp Asp Ala Asn Ala Leu His Tyr Ala Ala Ala
             20                  25                  30

Tyr Cys Asp Ser Lys Val Leu Thr Glu Leu Leu Gly Leu Glu Leu Ala
         35                  40                  45

Asn Leu Asn Leu Lys Asn Ser Arg Gly Tyr Thr Ala Leu His Leu Ala
     50                  55                  60

Ala Met Arg Arg Glu Pro Ala Ile Ile Met Cys Leu Leu Ser Lys Gly
 65                  70                  75                  80

Ala Val Ala Ser Gln Leu Thr Asp Asp Gly Arg Leu Ala Ser Asn Ile
                 85                  90                  95

Cys Arg Arg Leu Thr Arg Leu Lys Asp Tyr Asn Ala Lys Met Glu Gln
            100                 105                 110

Gly Gln Glu Ser Asn Lys Asp Arg Met Cys Ile Asp Ile Leu Glu Arg
        115                 120                 125

Glu Met Met Arg Asn Pro Met Thr Ala Glu Asp Ser Val Thr Ser Pro
    130                 135                 140

Leu Leu Ala Asp Asp Leu His Met Lys Leu Ser Tyr Leu Glu Asn Arg
```

145 150 155 160

Val Gly Leu Ala Gln
165

<210> 13
<211> 2326
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (419)..(1954)

<400> 13
ggccgcgagc caaagcccct ggtttcctcg caactgcctc cccgcgattc cgtttgaccc 60

ccactgttct tctcccctac caccaccagg tcgccgtcgc ttccaatttc caaataattc 120

cctccactcc ggccgctcgc gaggatagaa aaggatttct ttttctctct ctctctctcc 180

ccctctctcc gagatccgtt tcccaaacag gcgggggggtc gaaagtgttt ggtactttgg 240

tttggggagc ttgtttgccg acgcggatct gcgtggagac gagcagaggg gggagcgccg 300

gaattgggtg gtttggcccg ggaggcgccg gaaagtgggg gagcctttgg attccccgaa 360

cccgccatgg tgatccggca cgagtagtag tggtggtggt ggtattagta gcagtgag 418

atg ccg gcg cgt agc gcg gtg gtg gta ata gcc atg gag ccc tcg tcg 466
Met Pro Ala Arg Ser Ala Val Val Val Ile Ala Met Glu Pro Ser Ser
1 5 10 15

tcc atc acc atc gcg tcg tcg tcc tcg tac ctc tcg aac ggg tct agc 514
Ser Ile Thr Ile Ala Ser Ser Ser Ser Tyr Leu Ser Asn Gly Ser Ser
20 25 30

ccg cgg tac aag atg gag gag ctc gtg ccg gga ggc cgc gtg ggg cgc 562
Pro Arg Tyr Lys Met Glu Glu Leu Val Pro Gly Gly Arg Val Gly Arg
35 40 45

gac gcc ttc ctg tcg ctg ctg ggt tac ctg tac acg ggc aag ctc cgg 610
Asp Ala Phe Leu Ser Leu Leu Gly Tyr Leu Tyr Thr Gly Lys Leu Arg
50 55 60

ccg gcg ccg gat gac gtg gtg tcc tgc gcc gac ccc atg tgc ccg cac 658
Pro Ala Pro Asp Asp Val Val Ser Cys Ala Asp Pro Met Cys Pro His
65 70 75 80

gac tcg tgc ccg ccg gcg atc agg ttc aac gtc gag caa atg tac gcg 706
Asp Ser Cys Pro Pro Ala Ile Arg Phe Asn Val Glu Gln Met Tyr Ala
85 90 95

gcg tgg gcg ttc aag atc acc gag ctc atc tcg ctg ttc cag cga cgg 754

```
Ala Trp Ala Phe Lys Ile Thr Glu Leu Ile Ser Leu Phe Gln Arg Arg
            100                 105                 110

ctt ctt aac ttc gtc gat aag act cta gta gaa gat gtt ctt cca att   802
Leu Leu Asn Phe Val Asp Lys Thr Leu Val Glu Asp Val Leu Pro Ile
        115                 120                 125

ctg caa gtt gct ttt cat tca gag ctg act cca gtg ctt gaa aaa tgt   850
Leu Gln Val Ala Phe His Ser Glu Leu Thr Pro Val Leu Glu Lys Cys
    130                 135                 140

att cgg aga att gca aga tca aat ctt gat aat gta tcg ttg gat aag   898
Ile Arg Arg Ile Ala Arg Ser Asn Leu Asp Asn Val Ser Leu Asp Lys
145                 150                 155                 160

gaa ctt cct cca gaa gtt gct gtt cag ata aaa gag att cgc caa aaa   946
Glu Leu Pro Pro Glu Val Ala Val Gln Ile Lys Glu Ile Arg Gln Lys
                165                 170                 175

tct cag cca aat gag ggt gac acc gtc att tca gac cct gta cat gag   994
Ser Gln Pro Asn Glu Gly Asp Thr Val Ile Ser Asp Pro Val His Glu
            180                 185                 190

aaa agg gtc aga aga atc cac agg gca ctg gat tct gat gat gtt gag   1042
Lys Arg Val Arg Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu
        195                 200                 205

ctt gtg aag ttg ctt ctt aac gaa tct gag atc acc ttg gat gat gcc   1090
Leu Val Lys Leu Leu Leu Asn Glu Ser Glu Ile Thr Leu Asp Asp Ala
    210                 215                 220

aat gca ttg cac tat gct gct gct tac tgt gat tcg aaa gtt gtt tcg   1138
Asn Ala Leu His Tyr Ala Ala Ala Tyr Cys Asp Ser Lys Val Val Ser
225                 230                 235                 240

gag ttg tta gac ttg aga ctt gcc aac ttg aat ttg aag aat tcg cgt   1186
Glu Leu Leu Asp Leu Arg Leu Ala Asn Leu Asn Leu Lys Asn Ser Arg
                245                 250                 255

gga tac acg gca ctc cat ctg gct gct atg agg aga gag cca gct att   1234
Gly Tyr Thr Ala Leu His Leu Ala Ala Met Arg Arg Glu Pro Ala Ile
            260                 265                 270

atc atg tgt ctc cta aac aaa gga gca gct gta tca caa ttg act gct   1282
Ile Met Cys Leu Leu Asn Lys Gly Ala Ala Val Ser Gln Leu Thr Ala
        275                 280                 285

gat ggc cag agt gca atg agt atc tgc cgg agg tta aca agg atg aaa   1330
Asp Gly Gln Ser Ala Met Ser Ile Cys Arg Arg Leu Thr Arg Met Lys
    290                 295                 300

gac tac aat aca aag atg gag caa ggc caa gag tca aac aaa gac aga   1378
Asp Tyr Asn Thr Lys Met Glu Gln Gly Gln Glu Ser Asn Lys Asp Arg
305                 310                 315                 320

tta tgt att gat ata tta gat agg gag atg ata agg aaa cct atg gca   1426
```

```
Leu Cys Ile Asp Ile Leu Asp Arg Glu Met Ile Arg Lys Pro Met Ala
                325                 330                 335

gtg gaa gat tct gtc acc tcg cct ttg ttg gct gac gat ctt cac atg   1474
Val Glu Asp Ser Val Thr Ser Pro Leu Leu Ala Asp Asp Leu His Met
            340                 345                 350

aag ctt ctc tac ctt gaa aac aga gtt gca ttt gca aga tta ttt ttt   1522
Lys Leu Leu Tyr Leu Glu Asn Arg Val Ala Phe Ala Arg Leu Phe Phe
        355                 360                 365

cct gca gaa gca aag gtt gca atg caa att gca caa gca gac acc aca   1570
Pro Ala Glu Ala Lys Val Ala Met Gln Ile Ala Gln Ala Asp Thr Thr
    370                 375                 380

cca gaa ttt ggc att gtt cct gca gct agc act tct gga aaa ttg aag   1618
Pro Glu Phe Gly Ile Val Pro Ala Ala Ser Thr Ser Gly Lys Leu Lys
385                 390                 395                 400

gaa gtc gat ctg aac gag aca cca gta aca caa aac aaa agg ctc cgt   1666
Glu Val Asp Leu Asn Glu Thr Pro Val Thr Gln Asn Lys Arg Leu Arg
                405                 410                 415

tca agg gtg gat gca ctc atg aaa aca gtt gag ctg gga cgt cgc tac   1714
Ser Arg Val Asp Ala Leu Met Lys Thr Val Glu Leu Gly Arg Arg Tyr
            420                 425                 430

ttc cct aac tgc tcg cag gtg ctc gac aaa ttt ctg gag gat gat ttg   1762
Phe Pro Asn Cys Ser Gln Val Leu Asp Lys Phe Leu Glu Asp Asp Leu
        435                 440                 445

ccc gat agt cct gat gca ctc gac ctc caa aat ggc act tct gat gag   1810
Pro Asp Ser Pro Asp Ala Leu Asp Leu Gln Asn Gly Thr Ser Asp Glu
    450                 455                 460

caa aat gtt aaa agg atg cgg ttc tgt gag tta aag gag gat gtg cgc   1858
Gln Asn Val Lys Arg Met Arg Phe Cys Glu Leu Lys Glu Asp Val Arg
465                 470                 475                 480

aag gca ttc agc aaa gac aga gct gat aat agc atg ttt tct atc ttg   1906
Lys Ala Phe Ser Lys Asp Arg Ala Asp Asn Ser Met Phe Ser Ile Leu
                485                 490                 495

tca tct tca tcg tca tct tcg cca cct ccc aag gtt gca aag aaa tga   1954
Ser Ser Ser Ser Ser Ser Ser Pro Pro Pro Lys Val Ala Lys Lys
            500                 505                 510

cagaagtttt gtaacaaatt tccgctcgtg atgttactgg gacaagagat atcgatcaat 2014

agacctgtat agtcttacag tggtataaca attagatatc gaagcttctt cgaatattag 2074

aaagtgctgt tctgggctgc actcagctgg tttatgggac ccatgcggtg aaactggcaa 2134

aagaaaacca gctgattaga grctccaaag cagtgtctct cgtgaatatg tttgtagcat 2194

tctgttttgt tcaggatggc tataatgata aaatcttttc aatagatata tagctaattg 2254
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tctcgtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2314

aaaaaaaaaa aa 2326

<210> 14
<211> 511
<212> PRT
<213> Oryza sativa

<400> 14

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Met Pro Ala Arg Ser Ala Val Val Val Ile Ala Met Glu Pro Ser Ser
  1               5                  10                  15
Ser Ile Thr Ile Ala Ser Ser Ser Ser Tyr Leu Ser Asn Gly Ser Ser
             20                  25                  30
Pro Arg Tyr Lys Met Glu Glu Leu Val Pro Gly Gly Arg Val Gly Arg
         35                  40                  45
Asp Ala Phe Leu Ser Leu Leu Gly Tyr Leu Tyr Thr Gly Lys Leu Arg
     50                  55                  60
Pro Ala Pro Asp Asp Val Val Ser Cys Ala Asp Pro Met Cys Pro His
 65                  70                  75                  80
Asp Ser Cys Pro Pro Ala Ile Arg Phe Asn Val Glu Gln Met Tyr Ala
                 85                  90                  95
Ala Trp Ala Phe Lys Ile Thr Glu Leu Ile Ser Leu Phe Gln Arg Arg
            100                 105                 110
Leu Leu Asn Phe Val Asp Lys Thr Leu Val Glu Asp Val Leu Pro Ile
        115                 120                 125
Leu Gln Val Ala Phe His Ser Glu Leu Thr Pro Val Leu Glu Lys Cys
    130                 135                 140
Ile Arg Arg Ile Ala Arg Ser Asn Leu Asp Asn Val Ser Leu Asp Lys
145                 150                 155                 160
Glu Leu Pro Pro Glu Val Ala Val Gln Ile Lys Glu Ile Arg Gln Lys
                165                 170                 175
Ser Gln Pro Asn Glu Gly Asp Thr Val Ile Ser Asp Pro Val His Glu
            180                 185                 190
Lys Arg Val Arg Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu
        195                 200                 205
Leu Val Lys Leu Leu Leu Asn Glu Ser Glu Ile Thr Leu Asp Asp Ala
    210                 215                 220
Asn Ala Leu His Tyr Ala Ala Ala Tyr Cys Asp Ser Lys Val Val Ser
225                 230                 235                 240
Glu Leu Leu Asp Leu Arg Leu Ala Asn Leu Asn Leu Lys Asn Ser Arg
                245                 250                 255
Gly Tyr Thr Ala Leu His Leu Ala Ala Met Arg Arg Glu Pro Ala Ile
            260                 265                 270
Ile Met Cys Leu Leu Asn Lys Gly Ala Ala Val Ser Gln Leu Thr Ala
        275                 280                 285
Asp Gly Gln Ser Ala Met Ser Ile Cys Arg Arg Leu Thr Arg Met Lys
    290                 295                 300
Asp Tyr Asn Thr Lys Met Glu Gln Gly Gln Glu Ser Asn Lys Asp Arg
305                 310                 315                 320
Leu Cys Ile Asp Ile Leu Asp Arg Glu Met Ile Arg Lys Pro Met Ala
                325                 330                 335
Val Glu Asp Ser Val Thr Ser Pro Leu Leu Ala Asp Asp Leu His Met
            340                 345                 350
```

```
Lys Leu Leu Tyr Leu Glu Asn Arg Val Ala Phe Ala Arg Leu Phe Phe
        355                 360                 365
Pro Ala Glu Ala Lys Val Ala Met Gln Ile Ala Gln Ala Asp Thr Thr
    370                 375                 380
Pro Glu Phe Gly Ile Val Pro Ala Ala Ser Thr Ser Gly Lys Leu Lys
385                 390                 395                 400
Glu Val Asp Leu Asn Glu Thr Pro Val Thr Gln Asn Lys Arg Leu Arg
                405                 410                 415
Ser Arg Val Asp Ala Leu Met Lys Thr Val Glu Leu Gly Arg Arg Tyr
            420                 425                 430
Phe Pro Asn Cys Ser Gln Val Leu Asp Lys Phe Leu Glu Asp Asp Leu
        435                 440                 445
Pro Asp Ser Pro Asp Ala Leu Asp Leu Gln Asn Gly Thr Ser Asp Glu
    450                 455                 460
Gln Asn Val Lys Arg Met Arg Phe Cys Glu Leu Lys Glu Asp Val Arg
465                 470                 475                 480
Lys Ala Phe Ser Lys Asp Arg Ala Asp Asn Ser Met Phe Ser Ile Leu
                485                 490                 495
Ser Ser Ser Ser Ser Ser Ser Pro Pro Pro Lys Val Ala Lys Lys
            500                 505                 510
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<210> 15
<211> 1565
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)..(1263)

<400> 15

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ggc gcc ttc cca cca gct cgg gcg gga ggc ctc ctc ctc ctc ctc ctc   48
Gly Ala Phe Pro Pro Ala Arg Ala Gly Gly Leu Leu Leu Leu Leu Leu
  1               5                  10                  15

ctc gcc gag ctc acc aac ctc ttc cag cgg cgt ctc ctt gat gtc ctt   96
Leu Ala Glu Leu Thr Asn Leu Phe Gln Arg Arg Leu Leu Asp Val Leu
             20                  25                  30

gat aag gtt gaa gta gat aac ctt cta ttg atc tta tct gtt gcc aac   144
Asp Lys Val Glu Val Asp Asn Leu Leu Leu Ile Leu Ser Val Ala Asn
         35                  40                  45

tta tgc aac aaa tct tgc atg aaa ctg ctt gaa aga tgc ctt gat atg   192
Leu Cys Asn Lys Ser Cys Met Lys Leu Leu Glu Arg Cys Leu Asp Met
     50                  55                  60

gta gtc cgg tca aac ctt gac atg att act ctt gag aag tca ttg cct   240
Val Val Arg Ser Asn Leu Asp Met Ile Thr Leu Glu Lys Ser Leu Pro
 65                  70                  75                  80

cca gat gtt atc aag cag att att gat gca cgc cta agc ctc gga tta   288
Pro Asp Val Ile Lys Gln Ile Ile Asp Ala Arg Leu Ser Leu Gly Leu
                85                  90                  95
```

```
att tca cca gaa aac aag gga ttt cct aac aaa cat gtg agg agg ata   336
Ile Ser Pro Glu Asn Lys Gly Phe Pro Asn Lys His Val Arg Arg Ile
            100                 105                 110

cac aga gcc ctt gac tct gac gat gta gag cta gtc agg atg ctg ctc   384
His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu
        115                 120                 125

act gaa gga cag aca aat ctt gat gat gcg ttt gca ctg cac tac gcc   432
Thr Glu Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala
    130                 135                 140

gtc gaa cat tgt gac tcc aaa att aca acc gag ctt ttg gat ctc gca   480
Val Glu His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Leu Ala
145                 150                 155                 160

ctt gca gat gtt aat cat aga aac cca aga ggt tat act gtt ctt cac   528
Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His
                165                 170                 175

att gct gcg agg cga aga gag cct aaa atc att gtc tcc ctt tta acc   576
Ile Ala Ala Arg Arg Arg Glu Pro Lys Ile Ile Val Ser Leu Leu Thr
            180                 185                 190

aag ggg gct cga cca gca gat gtt aca ttc gat ggg aga aaa gcg gtt   624
Lys Gly Ala Arg Pro Ala Asp Val Thr Phe Asp Gly Arg Lys Ala Val
        195                 200                 205

caa atc tca aaa aga cta aca aaa caa ggg gat tac ttt ggg gtt acc   672
Gln Ile Ser Lys Arg Leu Thr Lys Gln Gly Asp Tyr Phe Gly Val Thr
    210                 215                 220

gaa gaa gga aaa cct tct cca aaa gat agg tta tgt att gaa ata ctg   720
Glu Glu Gly Lys Pro Ser Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu
225                 230                 235                 240

gag caa gct gaa aga agg gac cca caa ctc gga gaa gca tca gtt tct   768
Glu Gln Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Val Ser
                245                 250                 255

ctt gca atg gca ggt gag agt cta cga gga agg ttg ctg tat ctt gaa   816
Leu Ala Met Ala Gly Glu Ser Leu Arg Gly Arg Leu Leu Tyr Leu Glu
            260                 265                 270

aac cga gtt gct ttg gcg agg att atg ttt ccg atg gag gca aga gta   864
Asn Arg Val Ala Leu Ala Arg Ile Met Phe Pro Met Glu Ala Arg Val
        275                 280                 285

gca atg gat att gct caa gtg gat gga act ttg gaa ttt aac ctg ggt   912
Ala Met Asp Ile Ala Gln Val Asp Gly Thr Leu Glu Phe Asn Leu Gly
    290                 295                 300

tct ggt gca aat cca cct cct gaa aga caa cgg aca act gtt gat cta   960
Ser Gly Ala Asn Pro Pro Pro Glu Arg Gln Arg Thr Thr Val Asp Leu
305                 310                 315                 320
```

```
aat gaa agt cct ttc ata atg aaa gaa gaa cac tta gct cgg atg acg   1008
Asn Glu Ser Pro Phe Ile Met Lys Glu Glu His Leu Ala Arg Met Thr
                325                 330                 335

gca ctc tcc aaa aca gtg gag ctc ggg aaa cgc ttt ttc ccg cga tgt   1056
Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys
            340                 345                 350

tcg aac gtg ctc gac aag atc atg gat gat gaa act gat ccg gtt tcc   1104
Ser Asn Val Leu Asp Lys Ile Met Asp Asp Glu Thr Asp Pro Val Ser
        355                 360                 365

ctc gga aga gac acg tcc gcg gag aag agg aag agg ttt cat gac ctg   1152
Leu Gly Arg Asp Thr Ser Ala Glu Lys Arg Lys Arg Phe His Asp Leu
    370                 375                 380

cag gat gtt ctt cag aag gca ttc cac gag gac aag gag gag aat gac   1200
Gln Asp Val Leu Gln Lys Ala Phe His Glu Asp Lys Glu Glu Asn Asp
385                 390                 395                 400

agg tcg ggg ctc tcg tcg tcg tcg tca tcg aca tcg atc ggg gcc att   1248
Arg Ser Gly Leu Ser Ser Ser Ser Ser Ser Thr Ser Ile Gly Ala Ile
                405                 410                 415

cga cca agg aga tga acaccattgc tcccaaatag ttgccatatt gatagctaac   1303
Arg Pro Arg Arg
            420

tgtcctcctg gagctactca cctgatggtt gccttctgtc aattgccccc caaatatatt 1363

ctcaatggtt taggcttgta cagtattagt tcttacagct attgccccgt caattgtgaa 1423

acgcagaagt ttcactagtg cttgtactcg aggtgtaata caagtgcttg aattttgagt 1483

tgtacttgga atttccaggt ggtttgctcg taaaaatgag atgatttctt ggctcccaaa 1543

aaaaaaaaaa aaaaaaaaaa aa                                          1565
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<210> 16
<211> 420
<212> PRT
<213> Oryza sativa

<400> 16

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Gly Ala Phe Pro Pro Ala Arg Ala Gly Gly Leu Leu Leu Leu Leu Leu
  1               5                  10                  15
Leu Ala Glu Leu Thr Asn Leu Phe Gln Arg Arg Leu Leu Asp Val Leu
            20                  25                  30
Asp Lys Val Glu Val Asp Asn Leu Leu Leu Ile Leu Ser Val Ala Asn
        35                  40                  45
Leu Cys Asn Lys Ser Cys Met Lys Leu Leu Glu Arg Cys Leu Asp Met
    50                  55                  60
Val Val Arg Ser Asn Leu Asp Met Ile Thr Leu Glu Lys Ser Leu Pro
65                  70                  75                  80
```

```
Pro Asp Val Ile Lys Gln Ile Ile Asp Ala Arg Leu Ser Leu Gly Leu
                85                  90                  95
Ile Ser Pro Glu Asn Lys Gly Phe Pro Asn Lys His Val Arg Arg Ile
            100                 105                 110
His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu
        115                 120                 125
Thr Glu Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala
    130                 135                 140
Val Glu His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Leu Ala
145                 150                 155                 160
Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His
                165                 170                 175
Ile Ala Ala Arg Arg Arg Glu Pro Lys Ile Ile Val Ser Leu Leu Thr
            180                 185                 190
Lys Gly Ala Arg Pro Ala Asp Val Thr Phe Asp Gly Arg Lys Ala Val
        195                 200                 205
Gln Ile Ser Lys Arg Leu Thr Lys Gln Gly Asp Tyr Phe Gly Val Thr
    210                 215                 220
Glu Glu Gly Lys Pro Ser Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu
225                 230                 235                 240
Glu Gln Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Val Ser
                245                 250                 255
Leu Ala Met Ala Gly Glu Ser Leu Arg Gly Arg Leu Leu Tyr Leu Glu
            260                 265                 270
Asn Arg Val Ala Leu Ala Arg Ile Met Phe Pro Met Glu Ala Arg Val
        275                 280                 285
Ala Met Asp Ile Ala Gln Val Asp Gly Thr Leu Glu Phe Asn Leu Gly
    290                 295                 300
Ser Gly Ala Asn Pro Pro Pro Glu Arg Gln Arg Thr Thr Val Asp Leu
305                 310                 315                 320
Asn Glu Ser Pro Phe Ile Met Lys Glu Glu His Leu Ala Arg Met Thr
                325                 330                 335
Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys
            340                 345                 350
Ser Asn Val Leu Asp Lys Ile Met Asp Asp Glu Thr Asp Pro Val Ser
        355                 360                 365
Leu Gly Arg Asp Thr Ser Ala Glu Lys Arg Lys Arg Phe His Asp Leu
    370                 375                 380
Gln Asp Val Leu Gln Lys Ala Phe His Glu Asp Lys Glu Glu Asn Asp
385                 390                 395                 400
Arg Ser Gly Leu Ser Ser Ser Ser Ser Ser Thr Ser Ile Gly Ala Ile
                405                 410                 415
Arg Pro Arg Arg
            420
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<210> 17
<211> 2446
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (148)..(1977)

<220>
<221> misc_feature
<222> (1)..(2446)
<223> n = a, t, c, or g

<400> 17
ggccgacgcg gccctgcgtg gtgccaaaca aagcctgccc gaattgcgca gttcggccgg 60

gagagaccaa aaggcagcct ccccccttttg ccttcacaca tggtggtccg gctctagggc 120

cctttcgcct cgtgcttggc ggcggtg atg gag ccg tcg tcg tcc atc acg ttc 174
Met Glu Pro Ser Ser Ser Ile Thr Phe
1 5

gcc tcc tcg tcg tcc tac ctg tcc aac ggc tcc agc ccc tgc tcc gtc 222
Ala Ser Ser Ser Ser Tyr Leu Ser Asn Gly Ser Ser Pro Cys Ser Val
10 15 20 25

gct ctg gcg cca ctg ccc gcg gcg gac ggg tgg gga ggg ggt ggt gga 270
Ala Leu Ala Pro Leu Pro Ala Ala Asp Gly Trp Gly Gly Gly Gly Gly
30 35 40

ggg gga ggg agc agc agc agc gtc gag gct gtg agc ctg aat cgc ctc 318
Gly Gly Gly Ser Ser Ser Ser Val Glu Ala Val Ser Leu Asn Arg Leu
45 50 55

agc aac aac ctc gag cgc ctc ctc ctc gat tct gaa ctc gac tgc agc 366
Ser Asn Asn Leu Glu Arg Leu Leu Leu Asp Ser Glu Leu Asp Cys Ser
60 65 70

gac gcc gac gtc gac atg gcg gac ggc ggg ccg ccc atc ccc gtc cac 414
Asp Ala Asp Val Asp Met Ala Asp Gly Gly Pro Pro Ile Pro Val His
75 80 85

cgc tgc atc ctc gcc gcg cgc agc ccc ttc ttc cac gac ctc ttc cgc 462
Arg Cys Ile Leu Ala Ala Arg Ser Pro Phe Phe His Asp Leu Phe Arg
90 95 100 105

gcc cgc ggg agc cgc agt gat ggg gca gtc acc gcc tcc gcc tcc gcc 510
Ala Arg Gly Ser Arg Ser Asp Gly Ala Val Thr Ala Ser Ala Ser Ala
110 115 120

tcc gcc acc agt ggc gga gcg gga ggg gat gtg acc ggg agg ccg cag 558
Ser Ala Thr Ser Gly Gly Ala Gly Gly Asp Val Thr Gly Arg Pro Gln
125 130 135

tac aag atg gag gac ctc gtc cca ggt ggc cgt gtt ggt cgc gag gcc 606
Tyr Lys Met Glu Asp Leu Val Pro Gly Gly Arg Val Gly Arg Glu Ala
140 145 150

ttc ctg gcg ttc atg ggg tac ctc tac acc ggc agg ctc cgg ccc gcg 654
Phe Leu Ala Phe Met Gly Tyr Leu Tyr Thr Gly Arg Leu Arg Pro Ala
155 160 165

cca ctg gac gtg gtg tca tgt gct gat ctt gtg tgc ccg cac gac tcg 702
Pro Leu Asp Val Val Ser Cys Ala Asp Leu Val Cys Pro His Asp Ser

```
170                 175                 180                 185

tgc cca ccg gcc atc agg ttc gcc gtc gag ctc atg tac gcg gcg tgg   750
Cys Pro Pro Ala Ile Arg Phe Ala Val Glu Leu Met Tyr Ala Ala Trp
                190                 195                 200

acc ttc agg atc ccc gag ctc atg tcg ctg ttc cag cga cgg ctt atg   798
Thr Phe Arg Ile Pro Glu Leu Met Ser Leu Phe Gln Arg Arg Leu Met
            205                 210                 215

aac ttt atc gac aag act cta gct gaa gac gtc ctg cct atc ttg caa   846
Asn Phe Ile Asp Lys Thr Leu Ala Glu Asp Val Leu Pro Ile Leu Gln
        220                 225                 230

gtt gcc ttc cac tca gag ctt act caa gtg cgt gga aaa tgt gtt caa   894
Val Ala Phe His Ser Glu Leu Thr Gln Val Arg Gly Lys Cys Val Gln
    235                 240                 245

agg att gca aga tca gat ctt gat att atg tct ttg gat aag gaa ctc   942
Arg Ile Ala Arg Ser Asp Leu Asp Ile Met Ser Leu Asp Lys Glu Leu
250                 255                 260                 265

cct cca gaa att gct gat gag ata aaa aaa atc cga cag aaa tct tcc   990
Pro Pro Glu Ile Ala Asp Glu Ile Lys Lys Ile Arg Gln Lys Ser Ser
                270                 275                 280

cca att gat ggt gac acc atc att tcg gac cct gtt cac gag aaa aga   1038
Pro Ile Asp Gly Asp Thr Ile Ile Ser Asp Pro Val His Glu Lys Arg
            285                 290                 295

gta aga aga atc cac agg gca ctg gat tct gat gat gtt gaa ctt gtg   1086
Val Arg Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Val
        300                 305                 310

aag ttg ctt ctt aat gag tct gaa atc acc cta gac gac gcc aac gca   1134
Lys Leu Leu Leu Asn Glu Ser Glu Ile Thr Leu Asp Asp Ala Asn Ala
    315                 320                 325

ttg cat tat gct gca gct tac tgc gat tct aaa gtt ctt aca gag ttg   1182
Leu His Tyr Ala Ala Ala Tyr Cys Asp Ser Lys Val Leu Thr Glu Leu
330                 335                 340                 345

tta ggc ctg gaa ctt gcc aac ttg aat ttg aag aac agt cgt ggg tac   1230
Leu Gly Leu Glu Leu Ala Asn Leu Asn Leu Lys Asn Ser Arg Gly Tyr
                350                 355                 360

aca gca ctc cac cta gct gct atg agg aga gaa cca gct att att atg   1278
Thr Ala Leu His Leu Ala Ala Met Arg Arg Glu Pro Ala Ile Ile Met
            365                 370                 375

tgt ctc tta agc aaa gga gca gtg gcg tcg caa ttg aca gat gat ggc   1326
Cys Leu Leu Ser Lys Gly Ala Val Ala Ser Gln Leu Thr Asp Asp Gly
        380                 385                 390

cgc ctt gca agt aat att tgt cga aga tta aca aga cta aaa gat tac   1374
Arg Leu Ala Ser Asn Ile Cys Arg Arg Leu Thr Arg Leu Lys Asp Tyr
```

```
    395                 400                 405

aat gca aag atg gag cag ggc caa gag tca aat aaa gat agg atg tgc   1422
Asn Ala Lys Met Glu Gln Gly Gln Glu Ser Asn Lys Asp Arg Met Cys
410                 415                 420                 425

att gac atc cta gag agg gag atg atg agg aat cct atg aca gcg gaa   1470
Ile Asp Ile Leu Glu Arg Glu Met Met Arg Asn Pro Met Thr Ala Glu
                430                 435                 440

gat tca gtc acc tca cct tta ttg gct gat gat ctt cac atg aaa cta   1518
Asp Ser Val Thr Ser Pro Leu Leu Ala Asp Asp Leu His Met Lys Leu
            445                 450                 455

agc tac ctg gaa aat aga gtc gcg ttt gca aga cta ttc ttc cct gct   1566
Ser Tyr Leu Glu Asn Arg Val Ala Phe Ala Arg Leu Phe Phe Pro Ala
        460                 465                 470

gaa gcg aag gtt gcg atg caa att gcg caa gca gac atc aca cca gaa   1614
Glu Ala Lys Val Ala Met Gln Ile Ala Gln Ala Asp Ile Thr Pro Glu
    475                 480                 485

gtt ggt ggt ttt tct gca gca agt act tct ggt aaa ctg agg gaa gtc   1662
Val Gly Gly Phe Ser Ala Ala Ser Thr Ser Gly Lys Leu Arg Glu Val
490                 495                 500                 505

gat ctg aat gag acg cca gta aca aaa aac aaa agg cta cgt tcg agg   1710
Asp Leu Asn Glu Thr Pro Val Thr Lys Asn Lys Arg Leu Arg Ser Arg
                510                 515                 520

gtg gat gca cta gtg aaa aca gtg gaa ctg ggc cgt cgg tac ttc cca   1758
Val Asp Ala Leu Val Lys Thr Val Glu Leu Gly Arg Arg Tyr Phe Pro
            525                 530                 535

aac tgc tcg cag gtg ctc gac aaa ttc ttg gaa gat ggc ctg cct gat   1806
Asn Cys Ser Gln Val Leu Asp Lys Phe Leu Glu Asp Gly Leu Pro Asp
        540                 545                 550

ggc ctt gat gca ttc cag cag caa agc ggc acc cct gat gag caa cag   1854
Gly Leu Asp Ala Phe Gln Gln Gln Ser Gly Thr Pro Asp Glu Gln Gln
    555                 560                 565

gtg aag aag atg cgc ttc tgc gag gtg aag gag gac gtg cgc aaa gca   1902
Val Lys Lys Met Arg Phe Cys Glu Val Lys Glu Asp Val Arg Lys Ala
570                 575                 580                 585

tac agc aaa gac acg gcc gat aac agc atg ttt tca gcc ctg tcg tca   1950
Tyr Ser Lys Asp Thr Ala Asp Asn Ser Met Phe Ser Ala Leu Ser Ser
                590                 595                 600

aac tcc tca tcc tcg gcg atg aag tga aggtactgta acaggctgtt         1997
Asn Ser Ser Ser Ser Ala Met Lys
            605                 610

ttctggagat gtcaggacta aagagggatc gctggtcatg cgcatgtata gtgctcmcca 2057
```

tcgtgtaaaa ctgaatatga acatgaaaga aggccccaaa atagtagaag atgatatata 2117

ctttgctgga cttggagttt gttggagaag gctgtgccat cccattccag attcccaata 2177

tcaattttcc catgctggtt gtgaagacag agccgcggat catccagctc cgacgctatg 2237

catgcgtgca gcctgctgta tttgtttcgc atagctgcaa tacttatatg tttaataata 2297

gtactaggga gtagtaggtt attgaggctg tagcggaagt tggaacctmc cttaatgtaa 2357

gtgaaagggg ncagttgccc wttgtcgaat tgttgttatc aatacatagt tgattttcgb 2417

maaaaaaaaa aaaaaaaaaa aaaaaaaaa 2446

<210> 18
<211> 609
<212> PRT
<213> Triticum aestivum

<400> 18

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Met Glu Pro Ser Ser Ser Ile Thr Phe Ala Ser Ser Ser Ser Tyr Leu
  1               5                  10                  15
Ser Asn Gly Ser Ser Pro Cys Ser Val Ala Leu Ala Pro Leu Pro Ala
             20                  25                  30
Ala Asp Gly Trp Gly Gly Gly Gly Gly Gly Gly Gly Ser Ser Ser Ser
         35                  40                  45
Val Glu Ala Val Ser Leu Asn Arg Leu Ser Asn Asn Leu Glu Arg Leu
     50                  55                  60
Leu Leu Asp Ser Glu Leu Asp Cys Ser Asp Ala Asp Val Asp Met Ala
 65                  70                  75                  80
Asp Gly Gly Pro Pro Ile Pro Val His Arg Cys Ile Leu Ala Ala Arg
                 85                  90                  95
Ser Pro Phe Phe His Asp Leu Phe Arg Ala Arg Gly Ser Arg Ser Asp
            100                 105                 110
Gly Ala Val Thr Ala Ser Ala Ser Ala Ser Ala Thr Ser Gly Gly Ala
        115                 120                 125
Gly Gly Asp Val Thr Gly Arg Pro Gln Tyr Lys Met Glu Asp Leu Val
    130                 135                 140
Pro Gly Gly Arg Val Gly Arg Glu Ala Phe Leu Ala Phe Met Gly Tyr
145                 150                 155                 160
Leu Tyr Thr Gly Arg Leu Arg Pro Ala Pro Leu Asp Val Val Ser Cys
                165                 170                 175
Ala Asp Leu Val Cys Pro His Asp Ser Cys Pro Pro Ala Ile Arg Phe
            180                 185                 190
Ala Val Glu Leu Met Tyr Ala Ala Trp Thr Phe Arg Ile Pro Glu Leu
        195                 200                 205
Met Ser Leu Phe Gln Arg Arg Leu Met Asn Phe Ile Asp Lys Thr Leu
    210                 215                 220
Ala Glu Asp Val Leu Pro Ile Leu Gln Val Ala Phe His Ser Glu Leu
225                 230                 235                 240
Thr Gln Val Arg Gly Lys Cys Val Gln Arg Ile Ala Arg Ser Asp Leu
                245                 250                 255
Asp Ile Met Ser Leu Asp Lys Glu Leu Pro Pro Glu Ile Ala Asp Glu
            260                 265                 270
Ile Lys Lys Ile Arg Gln Lys Ser Ser Pro Ile Asp Gly Asp Thr Ile
```

```
        275                 280                 285
Ile Ser Asp Pro Val His Glu Lys Arg Val Arg Arg Ile His Arg Ala
    290                 295                 300
Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu Ser
305                 310                 315                 320
Glu Ile Thr Leu Asp Asp Ala Asn Ala Leu His Tyr Ala Ala Ala Tyr
                325                 330                 335
Cys Asp Ser Lys Val Leu Thr Glu Leu Leu Gly Leu Glu Leu Ala Asn
            340                 345                 350
Leu Asn Leu Lys Asn Ser Arg Gly Tyr Thr Ala Leu His Leu Ala Ala
        355                 360                 365
Met Arg Arg Glu Pro Ala Ile Ile Met Cys Leu Leu Ser Lys Gly Ala
    370                 375                 380
Val Ala Ser Gln Leu Thr Asp Asp Gly Arg Leu Ala Ser Asn Ile Cys
385                 390                 395                 400
Arg Arg Leu Thr Arg Leu Lys Asp Tyr Asn Ala Lys Met Glu Gln Gly
                405                 410                 415
Gln Glu Ser Asn Lys Asp Arg Met Cys Ile Asp Ile Leu Glu Arg Glu
            420                 425                 430
Met Met Arg Asn Pro Met Thr Ala Glu Asp Ser Val Thr Ser Pro Leu
        435                 440                 445
Leu Ala Asp Asp Leu His Met Lys Leu Ser Tyr Leu Glu Asn Arg Val
    450                 455                 460
Ala Phe Ala Arg Leu Phe Phe Pro Ala Glu Ala Lys Val Ala Met Gln
465                 470                 475                 480
Ile Ala Gln Ala Asp Ile Thr Pro Glu Val Gly Gly Phe Ser Ala Ala
                485                 490                 495
Ser Thr Ser Gly Lys Leu Arg Glu Val Asp Leu Asn Glu Thr Pro Val
            500                 505                 510
Thr Lys Asn Lys Arg Leu Arg Ser Arg Val Asp Ala Leu Val Lys Thr
        515                 520                 525
Val Glu Leu Gly Arg Arg Tyr Phe Pro Asn Cys Ser Gln Val Leu Asp
    530                 535                 540
Lys Phe Leu Glu Asp Gly Leu Pro Asp Gly Leu Asp Ala Phe Gln Gln
545                 550                 555                 560
Gln Ser Gly Thr Pro Asp Glu Gln Gln Val Lys Lys Met Arg Phe Cys
                565                 570                 575
Glu Val Lys Glu Asp Val Arg Lys Ala Tyr Ser Lys Asp Thr Ala Asp
            580                 585                 590
Asn Ser Met Phe Ser Ala Leu Ser Ser Asn Ser Ser Ser Ser Ala Met
        595                 600                 605
Lys
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<210> 19
<211> 1668
<212> DNA
<213> Triticum aestivum

<220>
<221> CDS
<222> (451)..(1668)

<400> 19
tgtacttgcg agcatttgaa acacataaaa ttacttttga taggttactt aaatatatgc 60

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aacttcgatg cagaggctgg ggtaataaaa tcttccattt tctatttttt gaaatacttg 120

ttgacagggc tgtaatcaaa ttgggttaat caatgtatgt gtttgtattc ttaaaatatt 180

acttatcaga ttagaccgtt tatgcgtcta tattcttatc aatccgtatg gctgtgtcga 240

gacttcggat ttttatgtat tttttagtga tgatatgctt ttccttctta gctttgtcat 300

actgagattt gtgttttaat aattctgact tcgctgcaga tgatttgccc gtgtatcgtt 360

tgatgctaac tctcgtcgac ttgctacttg taacagttct ctattgttct attgtttcat 420

gtttttgaga agcgagtact aacccatgtt atg ccc ttc ttt tcc atg cag cgg  474
                                Met Pro Phe Phe Ser Met Gln Arg
                                  1               5

cat ctc ctt gat ttc ctt gat aaa gtt gaa gtg gat aac ctt ccg ttg  522
His Leu Leu Asp Phe Leu Asp Lys Val Glu Val Asp Asn Leu Pro Leu
     10                  15                  20

atc tta tct gtt gca aac tta tgc aac aaa tct tgc gtg aaa ctg ttc  570
Ile Leu Ser Val Ala Asn Leu Cys Asn Lys Ser Cys Val Lys Leu Phe
 25                  30                  35                  40

gag aga tgc atg gag atg gta gtc cgg tca aat ctt gac atg att act  618
Glu Arg Cys Met Glu Met Val Val Arg Ser Asn Leu Asp Met Ile Thr
                 45                  50                  55

cta gag aaa gca ttg cct caa gat gtc atc aag caa att act gat tta  666
Leu Glu Lys Ala Leu Pro Gln Asp Val Ile Lys Gln Ile Thr Asp Leu
            60                  65                  70

cgg ata act ctt gga tta gct tca ccc gaa gac aat ggc ttt cct aac  714
Arg Ile Thr Leu Gly Leu Ala Ser Pro Glu Asp Asn Gly Phe Pro Asn
         75                  80                  85

aaa cac gta aga agg ata ctc aga gca ctt gat tct gat gat gtg gag  762
Lys His Val Arg Arg Ile Leu Arg Ala Leu Asp Ser Asp Asp Val Glu
     90                  95                 100

ctt gtc agg atg ctg ctc aca gaa ggg cag act aac ctt gat gat gca  810
Leu Val Arg Met Leu Leu Thr Glu Gly Gln Thr Asn Leu Asp Asp Ala
105                 110                 115                 120

ttt gca ttg cac tat gct gta gaa cac tgt gac tca aaa att aca aca  858
Phe Ala Leu His Tyr Ala Val Glu His Cys Asp Ser Lys Ile Thr Thr
                125                 130                 135

gaa ctt ctg gac atc gca ctt gcg gat gtt aat ctc aga aac cca aga  906
Glu Leu Leu Asp Ile Ala Leu Ala Asp Val Asn Leu Arg Asn Pro Arg
            140                 145                 150

ggt tat act gtt ctt cac atc gcc gct aag cgg aga gat cct aaa atc  954
Gly Tyr Thr Val Leu His Ile Ala Ala Lys Arg Arg Asp Pro Lys Ile
        155                 160                 165
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gtt gtc tcc ctt tta acc aaa ggt gcc cgg cct tca gat ttt aca ttt   1002
Val Val Ser Leu Leu Thr Lys Gly Ala Arg Pro Ser Asp Phe Thr Phe
    170                 175                 180

gat gga aga aaa gca gtt caa atc tca aag aga ctc aca aaa cat ggt   1050
Asp Gly Arg Lys Ala Val Gln Ile Ser Lys Arg Leu Thr Lys His Gly
185                 190                 195                 200

gat tat ttt ggg aat act gaa gaa gga aag ccg tct ccc aat gat aaa   1098
Asp Tyr Phe Gly Asn Thr Glu Glu Gly Lys Pro Ser Pro Asn Asp Lys
                205                 210                 215

tta tgc att gag ata ttg gag caa gct gaa aga agg gat cca caa ctt   1146
Leu Cys Ile Glu Ile Leu Glu Gln Ala Glu Arg Arg Asp Pro Gln Leu
            220                 225                 230

gga gaa gca tca ctt tct ctt gca ttg gct ggt gac tgt ctt cgt gga   1194
Gly Glu Ala Ser Leu Ser Leu Ala Leu Ala Gly Asp Cys Leu Arg Gly
        235                 240                 245

aag tta ctg tac ctt gaa aac cga gtt gct ttg gca agg ata atg ttt   1242
Lys Leu Leu Tyr Leu Glu Asn Arg Val Ala Leu Ala Arg Ile Met Phe
    250                 255                 260

cca att gag gca aga gta gca atg gac att gct caa gtg gat ggt act   1290
Pro Ile Glu Ala Arg Val Ala Met Asp Ile Ala Gln Val Asp Gly Thr
265                 270                 275                 280

ttg gaa ttt acc ctt ggt tct agt aca aat cca cct ctg gag ata aca   1338
Leu Glu Phe Thr Leu Gly Ser Ser Thr Asn Pro Pro Leu Glu Ile Thr
                285                 290                 295

acc gtt gat ctg aat gat act tct ttc aaa atg aag gag gaa cac tta   1386
Thr Val Asp Leu Asn Asp Thr Ser Phe Lys Met Lys Glu Glu His Leu
            300                 305                 310

gct cgg atg aga gcc ctc tcc aaa aca gtt gaa ctc ggc aaa cgt ttc   1434
Ala Arg Met Arg Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe
        315                 320                 325

ttc cca cgc tgt tca aat gtg ctg gac aag atc atg gac gat gaa cct   1482
Phe Pro Arg Cys Ser Asn Val Leu Asp Lys Ile Met Asp Asp Glu Pro
    330                 335                 340

gag ctg gct tcc ctc gga aga gat gca tcc tcc gag agg aag agg agg   1530
Glu Leu Ala Ser Leu Gly Arg Asp Ala Ser Ser Glu Arg Lys Arg Arg
345                 350                 355                 360

ttt cac gac ctg caa gat acg ctt ctg aag gcg ttc agc gag gac aag   1578
Phe His Asp Leu Gln Asp Thr Leu Leu Lys Ala Phe Ser Glu Asp Lys
                365                 370                 375

gag gag ttt aac aga acg aca acc ctt tca tct tcg tca tcg tcg acg   1626
Glu Glu Phe Asn Arg Thr Thr Thr Leu Ser Ser Ser Ser Ser Ser Thr
            380                 385                 390
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tcc act gta gca agg aac ttg gca ggt cga act agg aga tga          1668
Ser Thr Val Ala Arg Asn Leu Ala Gly Arg Thr Arg Arg
        395                 400                 405


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<400> 20
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  1               5                  10                  15
Val Glu Val Asp Asn Leu Pro Leu Ile Leu Ser Val Ala Asn Leu Cys
             20                  25                  30
Asn Lys Ser Cys Val Lys Leu Phe Glu Arg Cys Met Glu Met Val Val
         35                  40                  45
Arg Ser Asn Leu Asp Met Ile Thr Leu Glu Lys Ala Leu Pro Gln Asp
     50                  55                  60
Val Ile Lys Gln Ile Thr Asp Leu Arg Ile Thr Leu Gly Leu Ala Ser
 65                  70                  75                  80
Pro Glu Asp Asn Gly Phe Pro Asn Lys His Val Arg Arg Ile Leu Arg
                 85                  90                  95
Ala Leu Asp Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu Thr Glu
            100                 105                 110
Gly Gln Thr Asn Leu Asp Asp Ala Phe Ala Leu His Tyr Ala Val Glu
        115                 120                 125
His Cys Asp Ser Lys Ile Thr Thr Glu Leu Leu Asp Ile Ala Leu Ala
    130                 135                 140
Asp Val Asn Leu Arg Asn Pro Arg Gly Tyr Thr Val Leu His Ile Ala
145                 150                 155                 160
Ala Lys Arg Arg Asp Pro Lys Ile Val Val Ser Leu Leu Thr Lys Gly
                165                 170                 175
Ala Arg Pro Ser Asp Phe Thr Phe Asp Gly Arg Lys Ala Val Gln Ile
            180                 185                 190
Ser Lys Arg Leu Thr Lys His Gly Asp Tyr Phe Gly Asn Thr Glu Glu
        195                 200                 205
Gly Lys Pro Ser Pro Asn Asp Lys Leu Cys Ile Glu Ile Leu Glu Gln
    210                 215                 220
Ala Glu Arg Arg Asp Pro Gln Leu Gly Glu Ala Ser Leu Ser Leu Ala
225                 230                 235                 240
Leu Ala Gly Asp Cys Leu Arg Gly Lys Leu Leu Tyr Leu Glu Asn Arg
                245                 250                 255
Val Ala Leu Ala Arg Ile Met Phe Pro Ile Glu Ala Arg Val Ala Met
            260                 265                 270
Asp Ile Ala Gln Val Asp Gly Thr Leu Glu Phe Thr Leu Gly Ser Ser
        275                 280                 285
Thr Asn Pro Pro Leu Glu Ile Thr Thr Val Asp Leu Asn Asp Thr Ser
    290                 295                 300
Phe Lys Met Lys Glu Glu His Leu Ala Arg Met Arg Ala Leu Ser Lys
305                 310                 315                 320
Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Asn Val Leu
                325                 330                 335
Asp Lys Ile Met Asp Asp Glu Pro Glu Leu Ala Ser Leu Gly Arg Asp
            340                 345                 350
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Ala Ser Ser Glu Arg Lys Arg Arg Phe His Asp Leu Gln Asp Thr Leu
        355                 360                 365
Leu Lys Ala Phe Ser Glu Asp Lys Glu Glu Phe Asn Arg Thr Thr Thr
    370                 375                 380
Leu Ser Ser Ser Ser Ser Ser Thr Ser Thr Val Ala Arg Asn Leu Ala
385                 390                 395                 400
Gly Arg Thr Arg Arg
                405
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